

Life

Sixth Edition
The Science of Biology



PURVES • SADAVA • ORIAN • HELLER

Highlights of www.thelifewire.com

LIFE's Web Site!

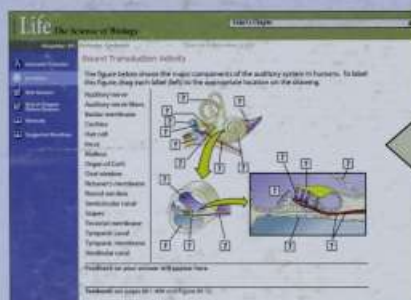


This main menu allows you to access all the features of the site by chapter or category.

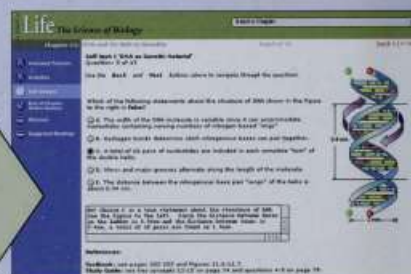
Tutorials clarify key concepts from the text. Most of the tutorials include animations, and all include a quiz with feedback for correct and incorrect answers.



In addition to tutorials, activities such as "Drag and Drop" exercises are provided for each chapter.



Self-test questions for each chapter of LIFE often reference key illustrations from the book. Extensive feedback is provided for each correct and incorrect answer with hot-links to specific passages in LIFE.



Additional Features of www.thelifewire.com

- **Flash Cards** test your knowledge of key terms and concepts.
- **Concept matching exercises** pair important concepts with definitions.
- **Glossary** provides definitions of almost 1600 key terms.

- **End of Chapter Online Quizzes** allow instructors to easily administer secure quizzes online.
- **Math for Life** helps you learn or reinforce basic quantitative skills.
- **Study Skills** provide class-tested practical advice on time management, test taking, note taking, and how to read a textbook.



Icons on these book pages indicate topics that are also covered as activities or tutorials on the Student Web Site.

CHAPTER	PAGE	TOPIC	CHAPTER	PAGE	TOPIC	CHAPTER	PAGE	TOPIC
1	9	The Hierarchy of Life	9	166	Fertilization and Meiosis Alternate in Sexual Reproduction	18	351	Summary
2	20	Chemical Bonds: Linking Atoms Together	9	168	Meiosis	19	354	The Human Defense System
2	20	Electron Orbitals	10	182	Homozygous or Heterozygous?	19	355	Blood Cells
2	31	Some Functional Groups Important to Living Systems	10	183	Meiosis Accounts for the Independent Assortment of Alleles	19	358	Interactions of Cells and Chemical Signals in Inflammation
3	34	Macromolecules: Giant Polymers	10	196	Summary	19	362	Structure of Immunoglobins
3	43	Glucose: From One Form to Another	11	206	DNA Replication	19	368	Phases of the Humoral and Cellular Immune Responses
3	47	Nucleotides Have Three Components	11	209	The Mechanism of DNA Replication	19	370	Heavy-Chain Gene Rearrangement and Splicing
3	47	Common and Distinguishing Characteristics of DNA and RNA	12	222	DNA Is Transcribed into RNA	19	375	Strategies to Combat HIV Reproduction
4	56	The Scale of Life	12	224	The Universal Genetic Code	20	380	Earth's Geological History
4	57	Looking at Cells with the Microscope	12	229	Translation: The Elongation Stage	20	382	The Continents Have Changed Position
4	60	An Animal Cell	13	252	The <i>lac</i> Operon: Transcription Is Induced by the Removal of a Repressor	21	400	The Hardy-Weinberg Equilibrium
4	61	A Plant Cell	13	253	The <i>trp</i> Operon: Transcription Is Repressed by the Binding of a Repressor	21	404	Natural Selection Produces Variable Results
4	67	Lysosomes Isolate Digestive Enzymes from the Cytoplasm	13	257	Summary	22	414	How Do New Species Arise?
5	80	The Fluid Mosaic Model	14	261	Eukaryotic mRNA Is Processed in the Nucleus and Exported to the Cytoplasm	22	423	Summary
5	84	Junctions Link Animal Cells Together	14	269	The Spliceosome, an RNA Splicing Machine	23	428	Reconstructing a Simple Phylogeny
5	85	Passive Processes of Membrane Transport	14	270	Potential Points of the Regulation of Gene Expression	23	436	Monophyletic, Polyphyletic, and Paraphyletic Taxa
5	88	Active Transport	14	271	The Initiation of Transcription in Eukaryotes	24	440	Amino Acid Sequence Alignment
5	91	Receptor-Mediated Endocytosis Is Highly Specific	15	284	A G Protein-Linked Receptor	25	457	Experiments Disproved the Spontaneous Generation of Life
6	102	Formation and Use of ATP	15	291	A Cascade of Reactions Leads to Altered Enzyme Activity	26	463	The Gram Stain and the Cell Wall
6	104	Enzyme and Substrate	15	293	Summary	26	466	The Nitrogen Cycle
6	104	Enzymes Lower the Activation Energy	16	295	Stages of Development	26	468	Lateral Gene Transfer Muddled the Phylogenetic Waters
6	109	Reversible Inhibition	16	306	A Gene Cascade Controls Pattern Formation in the <i>Drosophila</i> Embryo	27	486	Anatomy of a Paramecium
7	120	Glycolysis Converts Glucose to Pyruvate	17	321	DNA on a Chip	27	492	An Isomorphic Life Cycle
7	122	The Pyruvate Dehydrogenase Complex Catalyzes Pyruvate Oxidation	17	323	An Expression Vector Allows a Foreign Gene To Be Expressed in a Host Cell	27	493	A Haplontic Life Cycle
7	123	The Citric Acid Cycle	18	349	Two Approaches to Sequencing DNA	27	494	A Chloroplast Family Tree
7	127	A Chemiosmotic Mechanism Produces ATP				28	504	A Nontracheophyte Life Cycle
8	145	Chloroplasts Form ATP Chemiosmotically				28	511	Homospory and Heterospory
8	148	The Calvin-Benson Cycle				28	514	The Life Cycle of a Fern
8	149	Leaf Anatomy of C_3 and C_4 Plants				29	520	The Life Cycle of a Pine Tree
9	162	Mitosis						
9	162	The Mitotic Spindle Consists of Microtubules						

(Continued on inside back cover)



Life

The Science of Biology



Sixth Edition



Life

Sixth Edition

The Science of Biology

William K. Purves

*Emeritus, Harvey Mudd College
Claremont, California*

David Sadava

*The Claremont Colleges
Claremont, California*

Gordon H. Orians

*Emeritus, The University of Washington
Seattle, Washington*

H. Craig Heller

*Stanford University
Stanford, California*



Sinauer Associates, Inc.



W. H. Freeman and Company

The Cover

Giraffes (*Giraffa camelopardalis*) near Samburu, Kenya.
Photograph © BIOS/Peter Arnold, Inc.

The Opening Page

Soap yucca (*Yucca elata*), White Sands National Monument, New Mexico.
Photograph © David Woodfall/DRK PHOTO.

The Title Page

The endangered Florida panther (*Felis concolor coryi*).
Photograph © Thomas Kitchin/Tom Stack & Associates.

Life: The Science of Biology, Sixth Edition

Copyright © 2001 by Sinauer Associates, Inc. All rights reserved. This book may not be reproduced in whole or in part without permission.

Address editorial correspondence to:

Sinauer Associates, Inc., 23 Plumtree Road, Sunderland, Massachusetts 01375 U.S.A.
www.sinauer.com

Email: publish@sinauer.com

Address orders to:

VHPS/W. H. Freeman & Co. Order Department, 16365 James Madison Highway,
U.S. Route 15, Gordonsville, VA 22942 U.S.A.
www.whfreeman.com

Examination copy information: 1-800-446-8923
Orders: 1-888-330-8477

Library of Congress Cataloging-in-Publication Data

Life, the science of biology / William K. Purves...[et al.].--6th ed.
p. cm.

Includes index.

ISBN 0-7167-3873-2 (hardcover) -- ISBN 0-7167-4348-5 (Volume 1) --

ISBN 0-7167-4349-3 (Volume 2) -- ISBN 0-7167-4350-7 (Volume 3)

1. Biology I. Purves, William K. (William Kirkwood), 1934--

QH308.2 .L565 2000

570--dc21

00-048235

Printed in U.S.A.

Second Printing 2001 Courier Companies Inc.

This book is dedicated to the memory of Angeline Douvas

About the Authors



Gordon Orians

Craig Heller

Bill Purves

William K. Purves is Professor Emeritus of Biology as well as founder and former chair of the Department of Biology at Harvey Mudd College in Claremont, California. He received his Ph.D. from Yale University in 1959 under Arthur Galston. A fellow of the American Association for the Advancement of Science, Professor Purves has served as head of the Life Sciences Group at the University of Connecticut and as chair of the Department of Biological Sciences, University of California, Santa Barbara, where he won the Harold J. Pious Award for teaching excellence. His research interests focused on the chemical and physical regulation of plant growth and flowering. Professor Purves elected early retirement in 1995, after teaching introductory biology for 34 consecutive years, in order to turn his skills to writing and producing multimedia for introductory biology students. That year, he was awarded the Henry T. Mudd Prize as an outstanding member of the Harvey Mudd faculty or administration.

David Sadava is now responsible for Life's chapters on the cell (2-8), in addition to the chapters on genetics and heredity that he assumed in the previous edition. He is the Pritzker Family Foundation Professor of Biology at Claremont McKenna, Pitzer, and Scripps, three of the Claremont Colleges. Professor Sadava received his Ph.D. from the University of California, San Diego in 1972, and has been at Claremont ever since. The author of textbooks on cell biology and on plants, genes, and agriculture, Professor Sadava has done research in many areas of cell biology and biochemistry, ranging from developmental biology, to human diseases, to pharmacology. His current research concerns human lung cancer and its resistance to chemotherapy. Virtually all of the research articles he has published have undergraduates as coauthors. Professor Sadava has taught a variety of courses to both majors and nonmajors, including introductory biology, cell biology, genetics, molecular biology, and biochemistry, and he recently developed a new course on the biology of cancer. For the last 15 years, Professor Sadava has been a visiting professor in the Department of Molecular, Cellular, and Developmental Biology at the University of Colorado, Boulder, and is currently a visiting scientist at the City of Hope Medical Center.

Gordon H. Orians is Professor Emeritus of David Sadava Zoology at the University of Washington. He received his Ph.D. from the University of California, Berkeley in 1960 under Frank Pitelka. Professor Orians has been elected to the National Academy of Sciences and the American Academy of Arts and Sciences, and is a Foreign Fellow of the Royal Netherlands Academy of Arts and Sciences. He was President of the Organization for Tropical Studies, 1988-1994, and President of the Ecological Society of America, 1995-1996. He is chair of The Board on Environmental Studies and Toxicology of the National Research Council and a member of the board of directors of World Wildlife Fund-US. He is a recipient of the Distinguished Service Award of the American Institute of Biological Sciences. Professor Orians is a leading authority in ecology, conservation biology, and evolution, with research experience in behavioral ecology, plant-herbivore interactions, community structure, the biology of rare species, and environmental policy. He elected early retirement to be able to devote more time to writing and environmental policy activities.

H. Craig Heller is the Lorry Lokey/Business Wire Professor of Biological Sciences and Human Biology at Stanford University. He has served as Director of the popular interdisciplinary undergraduate program in Human Biology and is now Chairman of Biological Sciences. Professor Heller received his Ph.D. from Yale University in 1970 and did postdoctoral work at Scripps Institute of Oceanography on how the brain regulates body temperature of mammals. His current research focuses on the neurobiology of sleep and circadian rhythms. Professor Heller has done research on a great variety of animals ranging from hibernating squirrels to exercising athletes. He teaches courses on animal and human physiology and neurobiology.

Preface

Biologists' understanding of the living world is growing explosively. This isn't the world that the four authors of this book were born into. We never dreamed, as we began our research careers as freshly minted Ph.D.'s, that our science could move so rapidly. Biology has now entered the post-genomic era, allowing biologists and biomedical scientists to tackle once-unapproachable challenges. We are also at the threshold of some experiments that raise ethical concerns so great that we must stand back and participate with others in determining what is right to do and what is not.

The enormous growth and changes in biology create a special challenge for textbook authors. How can a biology textbook provide the basics, keep up with the exciting new discoveries, and not become overwhelming. The increasing bulk of

textbooks is of great concern to authors as well as to instructors and their students, who blanch at the prospect of too many pages, too many term papers, and too little sleep. Some reconsideration of what is essential and how that is best presented needs to be made if the proliferation of facts is not to obscure the fundamental principles.

Our major goals were brevity, emphasis on experiments, and better ways to help students learn

In writing the Sixth Edition of Life, we committed ourselves to reversing the pattern of ever increasing page lengths in new editions. We wanted a shorter book that brings the subject into sharper focus. We tried to achieve this by judicious reduction of detail, by more concise writing, and by more use of figures as primary teaching sources. It worked! Our efforts were successful. This edition is 200 pages shorter than its predecessor, yet it covers much exciting new material.

While working to tighten and shorten the text, we were also determined to retain and even increase our emphasis on how we know things, rather than just what we know. To that end, the Sixth Edition inaugurates 72 specially formatted figures that show how experiments, field observations, and comparative methods help biologists formulate and test hypotheses (the figure at right is an example). Another 26 figures highlight some of the many field and laboratory methods created to do this research. These Experiment and Research Methods illustrations are listed on the endpapers at the back of the book.

In the Fifth Edition, we introduced "balloon captions" that guide the reader through the illustrations (rather than having to wade through lengthy captions). This feature was widely applauded and we have worked to refine the balloons' effectiveness. In response to suggestions from users

EXPERIMENT

Question: Are all genes in a genome essential for cell survival? Can transposon mutagenesis be used to determine which genes are essential for cell survival?

METHOD

M. genitalium has 470 genes; only two are shown here.

Experiment 1

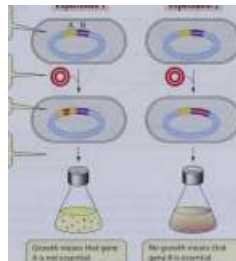
Experiment 2

A transposon inserts randomly into one gene...

...inactivating it.

The mutated bacterium is put into growth medium.

RESULTS



Conclusion: If each gene is inactivated in turn by mutagenesis, a "minimal essential genome" can be determined.

13.22 Using Transposon Mutagenesis to Determine the Minimal Genome

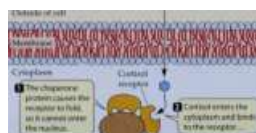
By inactivating genes one by one, scientists can determine which ones are essential for the cell's survival.

VIM

PREFACE

Signal (Cortisol)

Outside of cell



Cytoplasm

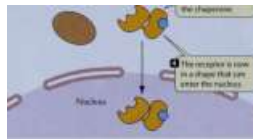
1) The chaperone protein causes the receptor to fold, so it cannot enter the nucleus.

Chaperone⁺

protein

isol enters the ~*=⁺ cytoplasm and binds 1 to the receptor...

Q ...causing the 1 receptor to change) shape and release Z⁺ \ the chaperone.



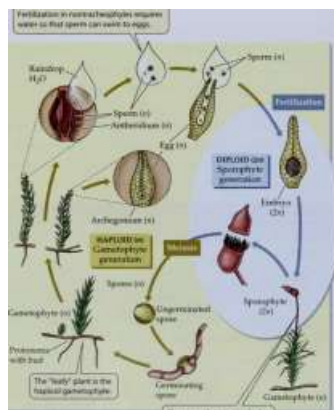
1S.9 A Cytoplasmic Receptor

The receptor for Cortisol is bound to a chaperone protein. Binding of the signal (which diffuses directly through the membrane) releases the chaperone and allows the receptor protein to enter the cell's nucleus, where it functions as a transcription factor.

Fertilization in nontracheophytes requires water so that sperm can swim to eggs.

Sperm (m)

Raindrop H₂O



Gametophyte (n)

The sporophyte is attached to and nutritionally dependent on the gametophyte.

|f7l⁺ 283 A Nontracheophyte Life Cycle

The life cycle of nontracheophytes, illustrated here by a moss, is dependent on an external source of liquid water. The visible green structure of nontracheophytes is the gametophyte; in nontracheophyte plants, the "leafy" structures are sporophytes.



of the Fifth Edition, in the Sixth Edition we now number many of the balloons, emphasizing the flow of the figure and making the sequence easier to follow (Figure 15.9 at left is an example).

This edition is accompanied by a comprehensive website, www.thelifewire.com (and an optional CD-ROM that contains the same material) that reinforces the content of every chapter. A key component of the website is a combination of animated tutorials and activities for each chapter, all of which include self-quizzes. Within each book chapter, this ^p icon refers students to a tutorial or an activity. An index of the icons begins in the front endpapers of the book. Figure 28.3 (left, below) shows a typical web icon placement.

As part of the ongoing challenge of keeping the writing and illustrations as clear as possible, we frequently employ bulleted lists. We think these lists will help students sort through what is, even after pruning, a daunting amount of material. And we have continued to provide plenty of interim summaries and bridges that link passages of text.

In all the introductory textbooks, the chapters end with summaries. In ours, we have organized the material within the chapter's main headings. In most cases, we tie key concepts to the figure (or figures) that illustrate it. For visual learners, this provides an efficient mode of reviewing the chapter.

From our many decades in the classroom, we know how important it is to motivate students. Each chapter begins with a brief description of some event, phenomenon, or idea that we hope will engage the reader while conveying a sense of the significance and purpose of the chapter's subject.

Evolution Continues to be the Dominant Theme

Evolution continues to be the most important of the themes that link our chapters and provide continuity. As we have written the various editions of the book, however, the emergence of genomics as a new paradigm in the late twentieth century has developed, revolutionizing most areas of biology. In this new century, understanding the workings of the genome is of paramount importance in almost any biological discussion.

In this edition, we have moved further toward updating the evolutionary theme to encompass the postgenomic era. Just two examples are the addition of a section on genomic evolution to our coverage of molecular evolution, and a section on "evo/devo" in the chapter on molecular biology of development. In addition, the chapters on the diversity of life reflect the vast changes in our understanding of systematics and phylogenetic relationships thanks to the genomic perspective.

In fact, each chapter of the book has undergone important changes.

PREFACE IX

The Seven Parts:

Content, Changes, and Themes

In Part One, The Cell, the emphasis in the discussions of biological molecules and thermodynamics has shifted more decisively toward biological aspects and away from pure chemistry. We have made our discussions of enzymes, cell respiration, and photosynthesis less detailed and more focused on the biological applications.

A major addition to Part Two, Information and Heredity, is a new chapter (Chapter 15) on cell signaling and communication, introduced at a place where the students have the necessary grounding in cell biology and molecular genetics. That chapter leads logically into an updated chapter (Chapter 16) on the molecular biology of development, which includes a new section on the intersection of evolutionary and developmental biology—"evo-devo" in the modern jargon. Several chapters incorporate the exciting new work in genomics of prokaryotes, humans, and other eukaryotes.

We have updated all the chapters in Part Three, Evolutionary Processes. In particular, Chapter 24 ("Molecular and Genomic Evolution") reflects the rapid advances in this exciting field. The section on genomic evolution (on pages 446–447) is brand new and includes Figure 24.9 (shown at right).

Part Four, The Evolution of Diversity, now reflects some exciting changes. The chapter on the protists—which can no longer be treated as a single "kingdom"—reflects the continuing uncertainty over the origin and early diversification of eukaryotes. The equally great uncertainty over prokaryote phylogeny, as we deal with the implications of extensive lateral transfer of genes, is evident in the chapter on prokaryote phyla.

We have extended the coverage of the evolution and diversity of plants to two chapters, and that of the animals to three. Recent findings stemming largely from molecular research have led to modifications of the phylogenies of angiosperms and of the animal kingdom. These changes are reflected in the many simplified "trees" that give a broad overview of systematic relationships. Key evolutionary events that separate and unite the different groups are highlighted with red "hot spots" (see Figure 33.1 at right).

We have rearranged Part Five, "The Biology of Flowering Plants," to allow Chapter 39 ("Plant Responses to Environmental Challenges") to serve as a capstone to the whole part, drawing together some of the major threads. We have added sections on hormones and photoreceptors discovered in recent years, and on their signal transduction pathways. The opening chapter (Chapter 34) on "The Flowering Plant Body" has an increased emphasis on meristems.

Part Six, The Biology of Animals, continues to be a broad, comparative treatment of animal physiology with an emphasis on mechanisms of control and regulation. Much new material has been added, including a major revision of Animal

H. influenzae

E. coli

Yeast

Drosophila

C. elegans (nematode)

Sea squirt

Putiferfish

Mouse

Human

0 25 50 75 100

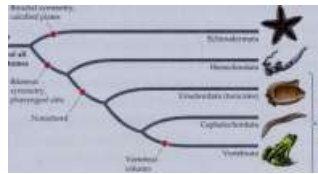
Number of genes x 1,000

24.9 Complex Organisms Have More Genes than Simpler Organisms

Genome sizes have been measured or estimated in a variety of organisms, ranging from single-celled prokaryotes to vertebrates.

Biradial symmetry calcified plates

Common marine ancestor of all deuterostomes



Bilateral symmetry, pharyngeal slits

Notochord

Vertebral column

33. 7 A Probable Deuterostomate Phylogeny

There are fewer major lineages and many fewer species of deuterostomes than of protostomes.

PREFACE

Development (Chapter 43) to complement and extend the earlier Chapter 16 (Development Differential Gene Expression). Some other new topics are the role of melatonin in photoperiodism, the role of leptin in the control of food intake, and the discovery in fruit flies of a gene that controls male mating behavior. The extensive coverage of the fast moving field of neurobiology has been substantially updated.

Throughout Part Seven, Ecology and Biogeography, we have added examples of experimental approaches to understanding the dynamics of ecological systems. Some of the examples illustrate the use of experimental and comparative methods. As before, we conclude the book with a chapter on conservation biology (Chapter 58), emphasizing the use of scientific principles to help preserve Earth's vast biological diversity.

There Are Many People to Thank

The reviewing process for Life, once a single pass at the stage of draft manuscript, has become an ongoing phenomenon. When the Fifth Edition was still young, we received critiques that influenced our work on this Sixth Edition. The two most penetrating ones came from Zach Gertz, then an undergraduate at Harvard, and Joseph Vanable, a veteran introductory biology professor at Purdue.

Next, still during the Fifth Edition run, 18 instructors recorded their suggestions for improvements in Life while teaching from the book. We call these reviews Diary Reviews. The third stage was the Manuscript Reviews. Seventy-three dedicated teachers and researchers read the first-draft chapters and gave us significant and cogent advice. Still another stage has been added to the process and it turned out to be invaluable. We are indebted to 16 Accuracy Reviewers, colleagues who carefully reviewed the almost final page proofs of each chapter to spot lingering errors or imprecisions in the text and art that inevitably escape our weary eyes. Finally, we appreciate the advice given by several experts who reviewed the animations and activities that our publishers developed for the student Web Site/ CD-ROM that accompanies this edition of Life. We thank all these reviewers and hope this new edition measures up to their expectations. They are listed after this Preface.

J/B Woolsey Associates has again worked closely with each of us to improve an already excellent art program. They helped to refine the very successful "balloon captions" that were introduced in the Fifth Edition. With their creative input we introduced the Experiment and Research Method illustrations found throughout the text.

James Funston joined us again as the developmental editor for the Sixth Edition. As always, James enforced a rigorous standard for clear writing and illustrating. And he contributed significantly to the process of shortening the book. Norma Roche also suggested cuts, and provided incisive copy editing from beginning to end. Her many astute queries often led to rewrites that enhanced the clarity of the presentation. From first draft to final pages, Susan McGlew was tireless in arranging for expert academic reviews of all of the chapters. Since the First Edition, we have profited immeasurably from the work of Carol Wigg, who again coordinated the pre-production process, including illustration editing and copy editing. She wrote many figure captions, suggested several of the chapter-opening stories, orchestrated the flow of the text and art, kept us mostly on schedule, enforced—sometimes with her red pen—the mandate to be concise, and what's more, did it all with good humor, even under pressure. David McIntyre, photo researcher, found many wonderful new photographs to enhance the learning experience and enliven the appearance of the book as a whole.

We again wish to thank the dedicated professionals in W. H. Freeman's marketing and sales group. Their enthusiasm has helped bring Life to a wider audience with each edition. We appreciate their continuing support and valuable input on ways to improve the book. A large share of Life's success is due to their efforts in this publishing partnership.

We have always respected Sinauer Associates for their outstanding list of biology books at all levels and we have enjoyed having them lead and assist us through yet another edition. Andy Sinauer has been the guiding spirit behind the development of Life since two of us first began to write the First Edition. Andy never ceases helping his authors to achieve our goals, while

remaining gentle but firm about his agendas. It has been a very satisfying experience for us to work with him yet again, and we look forward to a continuing association.

Bill Purves David Sadava Gordon Orians Craig Heller

November, 2000

Reviewers for the Sixth Edition

Diary Reviewers

Carla Barnwell, University of Illinois Greg Beaulieu, University of Victoria Gordon Fain, University of California,

Los Angeles Ruth Finkelstein, University

of California, Santa Barbara Steve Fisher, University of California,

Santa Barbara Alice Jacklet, SUNY, Albany Clare Hasenkampf, University of

Toronto, Scarborough Werner Heim, Colorado College David Hershey, Hyattsville, MD Hans-Willi Honegger, Vanderbilt

University Durrell Kapan, University of Texas,

Austin Cheryl Kerfeld, University of

California, Los Angeles Michael Martin, University of

Michigan, Ann Arbor Murray Nabors, Colorado State

University Ronald Poole, McGill University Nancy Sanders, Truman State

University Susan Smith, Massasoit Community

College Raymond White, City College of San

Francisco

Manuscript Reviewers

John Alcock, Arizona State University Allen V. Barker, University of

Massachusetts, Amherst Andrew R. Blaustein, Oregon State

University Richard Brusca, University of Arizona Matthew Buechner, University of

Kansas Warren Burggren, University of

North Texas Jung Choi, Georgia Institute of

Technology Andrew Clark, Pennsylvania State

University

Carla D'Antonio, University of California, Berkeley

Alan de Queiroz, University

of Colorado Michael Denbow, Virginia Tech Susan Dunford, University

of Cincinnati William Eickmeier, Vanderbilt

University John Endler, University of California,

Santa Barbara Gordon L. Fain, University of

California, Los Angeles Stu Feinstein, University of California,

Santa Barbara Danilo Fernando, SUNY, Syracuse Steve Fisher, University of California,

Santa Barbara Doug Futuyma, SUNY, Stony Brook Scott Gilbert, Swarthmore College Janice Glime, Michigan Technological

University Elizabeth Godrick, Boston University Robert Goodman, University of

Wisconsin, Madison Nancy Guild, University of Colorado Jessica Gurevitch, SUNY, Stony Brook Jeff Hardin, University of Wisconsin,

Madison Joseph Heilig, University of Colorado David Hershey, Hyattsville, MD Mark Johnston, Dalhousie University Walter Judd, University of Florida Thomas Kane, University of

Cincinnati Laura Katz, Smith College Elizabeth Kellogg, University of

Missouri, St. Louis Peter Krell, University of Guelph Thomas Kursar, University of Utah Wayne Maddison, University of Arizona William Manning, University of

Massachusetts, Amherst Michael Marcotrigiano, Smith College Lloyd Matsumoto, Rhode Island

College Stu Matz, The Evergreen State College D. Jeffrey Meldrum, Idaho State

University Mike Millay, Ohio University

(Southern Campus)

David Mindell, University of Michigan, Ann Arbor

Deborah Mowshowitz, Columbia University

Laura Olsen, University of Michigan, Ann Arbor

Guillermo Orti, University of

Nebraska Constance Parks, University of

Massachusetts, Amherst Jane Phillips, University of Minnesota Ronald Poole, McGill University Warren Porter, University of Wisconsin, Madison Thomas Poulson, University of

Illinois, Chicago Loren Rieseberg, Indiana University Ian Ross, University of California,

Santa Barbara Nancy Sanders, Truman State

University Paul Schroeder, Washington State

University Jim Shinkle, Trinity University Mitchell Sogin, Marine Biological

Laboratory, Woods Hole Wayne Sousa, University of California,

Berkeley Charles Staben, University of

Kentucky James Staley, University of

Washington Steve Stanley, The Johns Hopkins

University Barbara Stebbins-Boaz, Willamette

University Antony Stretton, University of

Wisconsin, Madison Steven Swoap, Williams College Gerald Thrush, California State

University, San Bernardino

Richard Tolman, Brigham Young

University Mary Tyler, University of Maine Michael Wade, Indiana University Bruce Walsh, University of Arizona Steven Wasserman, University of

California, San Diego Alex Weir, SUNY, Syracuse Mary Williams, Harvey Mudd College Jonathan Wright, Pomona College

REVIEWERS

Accuracy Reviewers

Andrew Clark, Pennsylvania State

University Joanne Ellzey, University of Texas,

El Paso Tejendra Gill, University of Houston,

University Park Paul Goldstein, University of Texas,

El Paso Laura Katz, Smith College Hans Landel, North Seattle

Community College Sandy Ligon, University of New

Mexico Peter Lortz, North Seattle Community

College

Roger Lumb, Western Carolina

University Coleman McCleneghan, Appalachian

State University Janie Milner, Santa Fe Community

College Zack Murrell, Appalachian State

University Ben Normark, University of

Massachusetts, Amherst Mike Silva, El Paso Community

College Phillip Snider, University of Houston,

University Park Steven Wasserman, University of

California, San Diego

Media Reviewers

Karen Bernd, Davidson College Mark Browning, Purdue University William Eldred, Boston University Joanne Ellzey, University of Texas,

El Paso Randall Johnson, University of

California, San Diego Coleman McCleneghan, Appalachian

State University Melissa Michael, University of Illinois Tom Pitzer, Florida International

University Kenneth Robinson, Purdue University

To the Student

Welcome to the study of life! In our student days—and ever since—we have enjoyed studying the fascinating and fast-changing field of biology and we hope that you will, too.

Getting the Most Out of the Book

There are a few things you can do to help you get the most from this book and from your course. For openers, read the book actively—don't just read passively, but do things that force you to think as you read. If we pose questions, stop and think about them. Ask questions of the text as you go. Do you understand what is being said? Does it relate to something you already know? Is it supported by experimental or other evidence? Does that evidence convince you? How does this passage fit into the chapter as a whole? Annotate the book—write down comments in the margins about things you don't understand, or about how one part relates to another, or even when you find an idea particularly interesting. People remember things they think about much better than they remember things they have read passively. Highlighting is passive; copying is drudge work; questioning and commenting are active and well worthwhile.

"Read" the illustrations actively too. You will find the balloon captions in the illustrations especially useful—they are there to guide you through the complexities of some topics and to highlight the major points.

The chapter summaries will help you quickly review the high points of what you have read. A summary identifies particular illustrations that you should study to help organize the material in your mind. Add concepts and details to the framework by reviewing the text. A way to review the material in slightly more detail after reading the chapter is to go back and look at the boldfaced terms. You can use the boldfaced terms to pose questions—and see if you can answer those questions. The boldfacing will probably be more useful on a second reading than on the first.

Use the "For Discussion" questions at the end of each chapter. These questions are usually open-ended and are intended to cause you to reflect on the material.

The glossary and the index can help you a great deal. When you are uncertain of the meaning of a term, check the glossary first—there are more than 1,500 definitions in it. If you don't find a term in the glossary, or if you want a more thorough discussion of the term, use the index to find where it's discussed.

The Web Site

Use the student Web Site/CD-ROM to help you understand some of the more detailed material and to help you sort out the information we have laid before you. An illustrated guide to the learning resources found on the Web Site/CD-ROM is in the front of this book. Pay particular attention to the activities and animated tutorials on key concepts, and to the self-quizzes. The self-quizzes provide extensive feedback for each correct and incorrect answer, and include hot-linked references to text

pages. If you'd like to pursue some topics in greater detail, you'll find a chapter-by-chapter annotated list of suggested readings. We have tried to choose readings from books and magazines, especially Scientific American, that should be available in your college library.

What If the Going Gets Tough?

Most students occasionally have difficulty in courses, including biology courses. If you find that you are slipping behind in the course, or if a particular topic is giving you an unreasonable amount of trouble, here are some useful steps you might take. First, the basics: attend class, take careful lecture notes, and read the textbook assignments. Second, note that one of the most important roles of studying is to discover what you don't know, so that you can do something about it. Use the index, the glossary, the chapter summaries, and the text itself to try to answer any questions you have and to help you organize the material. Make a habit of looking over your lecture notes within 24 hours of when you take them—find out right away what points are unclear, and get them straightened out in your mind. The web site can help by providing a different perspective.

If none of these self-help remedies does the trick, get help! Other students are often a good source of help, because they are dealing with the material at the same level as you are. Study groups can be very useful, as long as the participants are all committed to learning the material. Tutors are almost always helpful, as are faculty members. The main thing is to get help when you need it. It is not a good idea to be strong and silent and drift into a low grade.

But don't make the grade the point of this or any other course. You are in college to learn, to pursue interesting subjects, and to enjoy the subjects you are pursuing. We hope you'll enjoy the pursuit of biology.

Bill Purves David Sadava Gordon Orians Craig Heller

For the Student

Web Site/CD-ROM

Student Web Site at www.thelifewire.com

Life 6.0 CD-ROM (optionally bundled with the text)

The Web Site and CD-ROM each support the entire text, offering:

- ▶ Over 65 Animated Tutorials clarifying key topics from the text
- ▶ Activities, including flashcards for key terms and concepts, and drag-and-drop exercises
- ▶ Self-quizzes with extensive feedback, references to the Study Guide, and hot-linked references to Life: The Science of Biology, Sixth Edition
- ▶ Glossary of key terms and concepts
- ▶ End-of-chapter Online Quizzes (see "Online Quizzing" under "For the Instructor")
- ▶ Lifelines

Study Skills (Jerry Waldvogel, Clemson University) provides class-tested practical advice on time management, test-taking, note-taking, and how to read the textbook

Math for Life (Dany Adams, Smith College) helps students learn or reacquire basic quantitative skills

- ▶ Suggested Readings for further study

Order ISBN 0-7167-3874-0, Life 6.0 CD-ROM, or ISBN 0-7167-3875-9, Text/CD-ROM bundle

Study Guide

Christine Minor, Clemson University, Edward M. Dzialowski and Warren W. Burggren, University of North Texas, Lindsay Goodloe, Cornell University, and Nancy Guild, University of Colorado at Boulder.

For each chapter of the text, the study guide offers clearly defined learning objectives, summaries of key concepts, references to Life and to the student Web/CD-ROM, and review and exam-style self-test questions with answers and explanations.

Order ISBN 0-7167-3951-8

Lecture Notebook

This new tool presents black and white reproductions of all the Sixth Edition's line art and tables (more than 1000 images, with labels). The Notebook provides ample ruled spaces for note-taking. Order ISBN 0-7167-4449-X

For the Instructor

Instructor's Teaching Kit

This new comprehensive teaching tool (in a three-ring binder) combines:

1. Instructor's Manual

Erica Bergquist, Holyoke Community College The Manual includes:

- ▶ Chapter overviews
- ▶ Chapter outlines
- ▶ A "What's New" guide to the Sixth Edition
- ▶ All the bold-faced key terms from the text
- ▶ Key concepts and facts for each chapter
- ▶ Overviews of the animated tutorials from the Student Web Site/CD-ROM
- ▶ Custom lab ordering information (see "Custom Labs")

2. Enriched Lecture Notes, with diagrams Charles Herr, Eastern Washington University

3. A PowerPoint® Thumbnail Guide to the PowerPoint®

presentations on the Instructor's CD-ROM

Test Bank

Charles Herr, Eastern Washington University

The test bank, available in both computerized and printed formats, offers more than 4000 multiple-choice and sentence-completion questions.

The easy-to-use computerized test bank on CD-ROM includes Windows and Macintosh versions in a format that lets instructors add, edit, and resequence questions to suit their needs. From this same CD-ROM, instructors can access Diploma Online Testing from the Brownstone Research Group. Diploma allows instructors to easily create and administer secure exams over a network and over the Internet, with questions that incorporate multimedia and interactive exercises. More information about Diploma is available at <http://www.brownstone.net>

Online Quizzing

The online quizzing function is accessed via the Student Web Site at www.thelifewire.com. Using Question Mark's Perception, instructors can easily and securely quiz students online using multiple-choice questions for each text chapter and its media resources.

Instructor's Resource CD-ROM

The Instructor's Resource CD-ROM employs Presentation Manager and includes:

- ▶ All four-color line art and tables from the text (more than 1000 images), resized and reformatted to maximize large-hall projection
- ▶ More than 1500 photographic images, including electron micrographs, from the Biological Photo Service collection—all keyed to Life chapters
- ▶ More than 60 animations from the Student Web Site/CD-ROM
- ▶ Exceptional video microscopy from Jeremy Pickett-Heaps and others
- ▶ Chapter outlines and lecture notes from the Instructor's Teaching Kit in editable Microsoft® Word documents

PowerPoint® Presentations

The PowerPoint® slide set for Life follows the chapter summaries provided in the Instructor's Teaching Kit and can be used directly or customized. Each slide incorporates a figure from Life.

PowerPoint® Tutorials

QuickTime™ movies demonstrate how to use PowerPoint®.

Classroom Management

As a service for adopters using WebCT, we will provide a fully-loaded WebCourselet, including the instructor and student resources for this text. The files can then be customized to fit your specific course needs, or can be used as is. Course outlines, pre-built quizzes, activities, and a whole array of materials are included, eliminating hours of work for instructors interested in creating WebCT courses. For more information and a demo of the WebCourselet for this text, please visit our Web Site (<http://bfwpub.com/mediaroom/Index.html>) and click "WebCT".

Overhead Transparencies

The transparency set includes all four-color line art and tables from the text (more than 1000 images) in a convenient three-ring binder. Balloon captions (and some labels) are deleted to enhance projection and allow for classroom quizzing. Labels and images have been resized for maximum readability.

Slide Set

The slide set includes selected four-color figures from the text. Labels and images have been resized for maximum readability.

Laboratory Manuals

Biology in the Laboratory, Third Edition

Doris Helms, Robert Kosinski, and John Cummings, all of Clemson University

The revised edition of this popular lab manual, which includes a CD-ROM, is available to accompany the Sixth Edition of Life.

Order ISBN 0-7167-3146-0

Laboratory Outlines in Biology VI

Peter Abramoff and Robert G. Thomson, Marquette University

Order ISBN 0-7167-2633-5

The following manuals are available in a bound volume

or as separates:

Anatomy and Dissection of the Rat, Third Edition

Warren F. Walker, Jr., Oberlin College, and Dominique

Homberger, Louisiana State University

Order ISBN 0-7167-2635-1

Anatomy and Dissection of the Fetal Pig, Fifth Edition

Warren F. Walker, Jr., Oberlin College, and Dominique

Homberger, Louisiana State University

Order ISBN 0-7167-2637-8

Anatomy and Dissection of the Frog, Second Edition

Warren F. Walker, Jr., Oberlin College

Order ISBN 0-7167-2636-X

Custom Labs

Custom Publishing for Laboratory Manuals at www.custompub.whfreeman.com

With this custom publishing option, instructors can build and order customized lab manuals in just minutes, choosing material from Freeman's acclaimed biology laboratory manuals—lab-tested experiments that have been used successfully by hundreds of thousands of students. Instructors determine the manual's content (with the option to incorporate their own material or blank pages), table of contents or index styles, and cover design, and submit the order. A streamlined production process provides a quick turnaround to meet crucial deadlines.

Contents in Brief

1. An Evolutionary Framework for Biology 1

Part One THE CELL

2. Small Molecules: Structure and Behavior 17
3. Macromolecules: Their Chemistry and Biology 34
4. The Organization of Cells 55
5. Cellular Membranes 79
6. Energy, Enzymes, and Metabolism 95
7. Cellular Pathways That Harvest Chemical Energy 114
8. Photosynthesis: Energy from the Sun 136

Part Two INFORMATION AND HEREDITY

9. Chromosomes, the Cell Cycle, and Cell Division 155
10. Genetics: Mendel and Beyond 176
11. DNA and Its Role in Heredity 199
12. From DNA to Protein: Genotype to Phenotype 218
13. The Genetics of Viruses and Prokaryotes 239
14. The Eukaryotic Genome and Its Expression 259
15. Cell Signaling and Communication 279
16. Development: Differential Gene Expression 294
17. Recombinant DNA and Biotechnology 311
18. Molecular Biology and Medicine 331
19. Natural Defenses against Disease 353

Part Three EVOLUTIONARY PROCESSES

20. The History of Life on Earth 379
21. The Mechanisms of Evolution 395
22. Species and Their Formation 413
23. Reconstructing and Using Phylogenies 425
24. Molecular and Genomic Evolution 438
25. The Origin of Life on Earth 450

Part Four THE EVOLUTION OF DIVERSITY

26. Bacteria and Archaea: The Prokaryotic Domains 459
27. Protists and the Dawn of the Eukarya 476
28. Plants without Seeds: From Sea to Land 500

29. The Evolution of Seed Plants 516

30. Fungi: Recyclers, Killers, and Plant Partners 529
31. Animal Origins and Lophotrochozoans 543
32. Ecdysozoans: The Molting Animals 564
33. Deuterostome Animals 577

Part Five THE BIOLOGY OF FLOWERING PLANTS

34. The Plant Body 603
35. Transport in Plants 620
36. Plant Nutrition 634
37. Plant Growth Regulation 646
38. Reproduction in Flowering Plants 665
39. Plant Responses to Environmental Challenges 679

Part Six THE BIOLOGY OF ANIMALS

40. Physiology, Homeostasis, and Temperature Regulation 693
41. Animal Hormones 712
42. Animal Reproduction 732
43. Animal Development 752
44. Neurons and Nervous Systems 773
45. Sensory Systems 794
46. The Mammalian Nervous System: Structure and Higher Functions 814
47. Effectors: Making Animals Move 831
48. Gas Exchange in Animals 849
49. Circulatory Systems 866
50. Animal Nutrition 886
51. Salt and Water Balance and Nitrogen Excretion 910
52. Animal Behavior 925

Part Seven ECOLOGY AND BIOGEOGRAPHY

53. Behavioral Ecology 947
54. Population Ecology 959
55. Community Ecology 974
56. Ecosystems 991
57. Biogeography 1007
58. Conservation Biology 1030

Contents

1 An Evolutionary Framework for Biology 1

Organisms Have Changed over Billions of Years 1

Evolutionary Milestones 3

Life arises from nonlife 3

Cells form from molecules 3

Photosynthesis changes Earth's environment 4

Sex enhances adaptation 4

Eukaryotes are "cells within cells" 4

Multicellularity permits specialization of cells 5

Controlling internal environments becomes more complicated 5

Multicellular organisms undergo regulated growth 5

Speciation produces the diversity of life 6

The Hierarchy of Life 7

Biologists study life at different levels 9

Biological diversity is organized hierarchically 9

Asking and Answering "How?" and "Why?" 10

Hypothesis testing guides scientific research 10

Applying the hypothetico-deductive method 11

Experiments are powerful tools 12

Accepted scientific theories are based on many kinds of evidence 13

Not all forms of inquiry are scientific 13

Biology and Public Policy 14

Part One THE CELL



2 Small Molecules: Structure and Behavior 17

Atoms: The Constituents of Matter 17

An element is made up of only one kind of atom 18

The number of protons identifies the element 19

Isotopes differ in number of neutrons 19

Electron behavior determines chemical bonding 19

Chemical Bonds: Linking Atoms Together 20

Covalent bonds consist of shared pairs of electrons 20

Hydrogen bonds may form between molecules 23

Ions form bonds by electrical attraction 24

Polar and nonpolar substances interact best among themselves 24

Chemical Reactions: Atoms Change Partners 25

Water: Structure and Properties 26

Water has a unique structure and special properties 26

Most biological substances are dissolved in water 28

Acids, Bases, and the pH Scale 28

Acids donate H^+ , bases accept H^+ 28

Water is a weak acid 29

pH is the measure of hydrogen ion concentration 29

Buffers minimize pH change 30

The Properties of Molecules 30

Functional groups give specific properties to molecules 31

Isomers have different arrangements of the same atoms 32

3 Macromolecules: Their Chemistry and Biology 34

Macromolecules: Giant Polymers 34

Condensation Reactions 35

Proteins: Polymers of Amino Acids 36

Proteins are composed of amino acids 36

Peptide linkages covalently bond amino acids together 37

The primary structure of a protein is its amino acid sequence 38

The secondary structure of a protein requires hydrogen bonding 38

The tertiary structure of a protein is formed by bending and folding 39

The quaternary structure of a protein consists of subunits 40

The surfaces of proteins have specific shapes 40

Protein shapes are sensitive to the environment 41

Chaperonins help shape proteins 42

Carbohydrates: Sugars and Sugar Polymers 43

Monosaccharides are simple sugars 43
 Glycosidic linkages bond monosaccharides together 44
 Polysaccharides serve as energy stores or structural materials 45
 Chemically modified carbohydrates contain other groups 46

Nucleic Acids: Informational Macromolecules 47

The nucleic acids have characteristic structures and properties 47
 The uniqueness of a nucleic acid resides in its base sequence 48
 DNA is a guide to evolutionary relationships 49
 Nucleotides have other important roles in the cell 49

Lipids: Water-Insoluble Molecules 49

Fats and oils store energy 49
 Phospholipids form the core of biological membranes 51
 Carotenoids and steroids 51
 Some lipids are vitamins 52
 Wax coatings repel water 52

The Interactions of Macromolecules 53

4 The Organization of Cells 55

The Cell: The Basic Unit of Life 55

Cell size is limited by the surface area-to-volume ratio 55
 Microscopes are needed to visualize cells 56
 All cells are surrounded by a plasma membrane 58
 Cells show two organizational patterns 58

Prokaryotic Cells 58

All prokaryotic cells share certain features 58
 Some prokaryotic cells have specialized features 58

Eukaryotic Cells 59

Compartmentalization is the key to eukaryotic cell function 62

Organelles that Process Information 63

The nucleus stores most of the cell's DNA 63
 Ribosomes are the sites of protein synthesis 64

The Endomembrane System 64

The endoplasmic reticulum is a complex factory 64
 The Golgi apparatus stores, modifies, and packages proteins 65
 Lysosomes contain digestive enzymes 66

Organelles that Process Energy 67

Mitochondria are energy transformers 68
 Plastids photosynthesize or store materials 68
 Mitochondria and chloroplasts may have an endosymbiotic origin 70

Other Organelles 71

Peroxisomes house specialized chemical reactions 71
 Vacuoles are filled with water and soluble substances 71

The Cytoskeleton 72

Microfilaments function in support and movement 72
 Intermediate filaments are tough supporting elements 74
 Microtubules are long and hollow 74
 Microtubules power cilia and flagella 74

Extracellular Structures 76

The plant cell wall consists largely of cellulose 76
 Multicellular animals have elaborate extracellular matrices 76

5 Cellular Membranes 79

Membrane Composition and Structure 79

Lipids constitute the bulk of a membrane 79
 Membrane proteins are asymmetrically distributed 81
 Membrane carbohydrates are recognition sites 82

Cell Adhesion 82

Cell adhesion involves recognition proteins 83

Specialized Cell Junctions 84

Tight junctions seal tissues and prevent leaks 85
 Desmosomes hold cells together 85
 Gap junctions are a means of communication 85

Passive Processes of Membrane Transport 85



The physical nature of diffusion 85

Simple diffusion takes place through the membrane bilayer 86

Osmosis is the diffusion of water across membranes 86

Diffusion may be aided by channel proteins 87

Carrier proteins aid diffusion by binding substances 88

Active Transport 88

Active transport is directional 89

Primary and secondary active transport rely on different energy sources 89

Endocytosis and Exocytosis 90

Macromolecules and particles enter the cell by endocytosis 90

Receptor-mediated endocytosis is highly specific 91

Exocytosis moves materials out of the cell 91

Membranes Are Not Simply Barriers 92

Membranes Are Dynamic 92

6 Energy, Enzymes, and Metabolism 95

Energy and Energy Conversions 95

Energy changes are related to changes in matter 96

The first law: Energy is neither created nor destroyed 97

The second law: Not all energy can be used, and disorder tends to increase 97

Chemical reactions release or take up energy 99

Chemical equilibrium and free energy are related 100

ATP: Transferring Energy in Cells 100

ATP hydrolysis releases energy 100

ATP couples exergonic and endergonic reactions 101

Enzymes: Biological Catalysts 102

For a reaction to proceed, an energy barrier must be overcome 102

Enzymes bind specific reactant molecules 103

Enzymes lower the activation energy barrier but do not affect equilibrium 104

What are the chemical events at active sites of enzymes? 105

Substrate concentration affects reaction rate 105

Molecular Structure Determines Enzyme Function 106

The active site is specific to the substrate 106

An enzyme changes shape when it binds a substrate 106

To operate, some enzymes require added molecules 107

Metabolism and the Regulation of Enzymes 108

Metabolism is organized into pathways 108

Enzyme activity is subject to regulation 108

Allosteric enzymes have interacting subunits 109

Enzymes and their environment 111



7 Cellular Pathways That Harvest Chemical Energy 114

Obtaining Energy and Electrons from Glucose 114

Cells trap free energy while metabolizing glucose 114

Redox reactions transfer electrons and energy 115

The coenzyme NAD is a key electron carrier in redox reactions 116

An Overview: Releasing Energy from Glucose 116

Glycolysis: From Glucose to Pyruvate 118

The energy-investing reactions of glycolysis require ATP 118

The energy-harvesting reactions of glycolysis yield ATP and $\text{NADH} + \text{H}^+$ 119

Pyruvate Oxidation 122

The Citric Acid Cycle 122

The citric acid cycle produces two CO_2 molecules and reduced carriers 122

The Respiratory Chain: Electrons, Proton Pumping, and ATP 125

The respiratory chain transports electrons and releases energy 125

Active proton transport is followed by diffusion coupled to ATP synthesis 126

Fermentation: ATP from Glucose, without O_2 129

Some fermenting cells produce lactic acid and others produce alcohol 129

Contrasting Energy Yields 130

Metabolic Pathways 130

Catabolism and anabolism involve interconversions using carbon skeletons 131

Catabolism and anabolism are integrated 132

Regulating Energy Pathways 132

Allostery regulates metabolism 133

Evolution has led to metabolic efficiency 133

8 Photosynthesis: Energy from the Sun 136

Identifying Photosynthetic Reactants and Products 136

The Two Pathways of Photosynthesis: An Overview 137

Properties of Light and Pigments 138

Light comes in packets called photons 138

Absorption of a photon puts a pigment in an excited state 139

Light absorption and biological activity vary with wavelength 140

Photosynthesis uses chlorophylls and accessory pigments 141

Light Reactions: Light Absorption 142

Excited chlorophyll acts as a reducing agent 142

Electron Flow, Photophosphorylation and Reductions 143

Noncyclic electron flow produces ATP and NADPH 143

Cyclic electron flow produces ATP but no NADPH 144

Chemiosmosis is the source of ATP in photophosphorylation 145

Photosynthetic pathways are the products of evolution 146

Making Sugar from CO_2 : The Calvin-Benson Cycle 146

Isotope labeling experiments reveal the steps of Calvin-Benson cycle 146

The Calvin-Benson cycle is composed of three processes 147

Photorespiration and Its Evolutionary Consequences 148

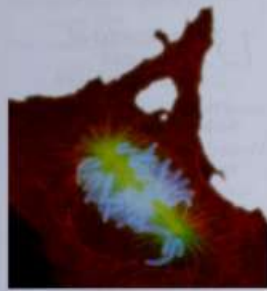
RuBP reacts with O_2 in photorespiration 148

Some plants have evolved systems to bypass photorespiration 149

CAM plants also use PEP carboxylase 151

Metabolic Pathways in Plants 151

Part Two INFORMATION AND HEREDITY



9 Chromosomes, the Cell Cycle, and Cell Division 155

Systems of Cell Reproduction 155

Prokaryotes divide by fission 155
Eukaryotic cells divide by mitosis
or meiosis 157

Interphase and the Control of Cell Division 157

Cyclins and other proteins signal
events in the cell cycle 158
Growth factors can stimulate cells
to divide 159

Eukaryotic Chromosomes 159

Chromatin consists of DNA and
proteins 160
Chromatin proteins organize the
DNA in chromosomes 160

Mitosis: Distributing Exact Copies of Genetic Information 160

The centrosomes determine the
plane of cell division 160
The spindle forms during
prophase 161
A prophase chromosome consists
of two chromatids 163
Chromosome movements are
highly organized 163
Nuclei re-form during telophase
164

Cytokinesis: The Division of the Cytoplasm 164

Reproduction: Sexual and Asexual 164

Reproduction by mitosis results in
genetic constancy 165
Reproduction by meiosis results in
genetic diversity 165
The number, shapes, and sizes of
the metaphase chromosomes
constitute the karyotype 167

Meiosis: A Pair of Nuclear Divisions 167

The first meiotic division reduces
the chromosome number 170
The second meiotic division sepa-
rates the chromatids 171
Meiosis leads to genetic diversity
172

Meiotic Errors 172

Aneuploidy can give rise to genet-
ic abnormalities 172
Polyploids can have difficulty in
cell division 173

Cell Death 173

10 Genetics: Mendel and Beyond 176

The Foundations of Genetics 176

Plant breeders showed that both
parents contribute equally to
inheritance 176
Mendel's discoveries were over-
looked for decades 177

Mendel's Experiments and the Laws of Inheritance 178

Mendel devised a careful research
plan 179
Mendel's Experiment 1 examined
a monohybrid cross 179
Mendel's first law says that alleles
segregate 180
Mendel verified his hypothesis by
performing a test cross 181
Mendel's second law says that
alleles of different genes assort
independently 182
Punnett squares or probability cal-
culations: A choice of methods
183
Mendel's laws can be observed in
human pedigrees 185

Alleles and Their Interactions 185

New alleles arise by mutation 186
Many genes have multiple alleles
186
Dominance is usually not com-
plete 186

In codominance, both alleles are
expressed 187

Some alleles have multiple pheno-
typic effects 187

Gene Interactions 188

Some genes alter the effects of
other genes 188
Hybrid vigor results from new
gene combinations and inter-
actions 188
Polygenes mediate quantitative
inheritance 189
The environment affects gene
action 189

Genes and Chromosomes 190

Linked genes are on the same
chromosome 190
Genes can be exchanged between
chromatids 191
Geneticists make maps of eukary-
otic chromosomes 192

Sex Determination and Sex- Linked Inheritance 193

Sex is determined in different
ways in different species 193
The X and Y chromosomes have
different functions 194
Genes on sex chromosomes are
inherited in special ways 195
Human beings display many sex-
linked characters 195

Non-Nuclear Inheritance 196

11 DNA and Its Role in Heredity 199

DNA: The Genetic Material 199

DNA from one type of bacterium
genetically transforms another
type 200
The transforming principle is
DNA 201
Viral replication experiments con-
firm that DNA is the genetic
material 201

The Structure of DNA 202

X-ray crystallography provided
clues to DNA structure 202
The chemical composition of DNA
was known 203
Watson and Crick described the
double helix 203
Four key features define DNA
structure 204
The double helical structure of
DNA is essential to its function
204

DNA Replication 206

Three modes of DNA replication appeared possible 206
 Meselson and Stahl demonstrated that DNA replication is semiconservative 207

The Mechanism of DNA Replication 209

DNA is threaded through a replication complex 209
 Small, circular DNA's replicate from a single origin, while large, linear DNA's have many origins 209
 Most DNA polymerases need a primer 210
 DNA polymerase III extends the new DNA strands 211
 The lagging strand is synthesized from Okazaki fragments 211

DNA Proofreading and Repair 212

Proofreading and repair mechanisms ensure that DNA replication is accurate 213
 DNA repair requires energy 214

Practical Applications of DNA Replication 214

The nucleotide sequence of DNA can be determined 214
 The polymerase chain reaction makes multiple copies of DNA 214

12 From DNA to Protein: Genotype to Phenotype 218**One Gene, One Polypeptide 218****DNA, RNA, and the Flow of Information 220**

RNA differs from DNA 220
 Information flows in one direction when genes are expressed 220
 RNA viruses modify the central dogma 221

Transcription: DNA-Directed RNA Synthesis 222

Initiation of transcription requires a promoter and an RNA polymerase 222
 RNA polymerase elongates the transcript 222
 Transcription terminates at particular base sequences 223

The Genetic Code 223

The genetic code is redundant but not ambiguous 224



Biologists broke the genetic code by using artificial messengers 224

Preparation for Translation: Linking RNA's, Amino Acids, and Ribosomes 225

Transfer RNA's carry specific amino acids and bind to specific codons 225
 Activating enzymes link the right tRNA's and amino acids 226
 The ribosome is the staging area for translation 226

Translation: RNA-Directed Polypeptide Synthesis 228

Translation begins with an initiation complex 228
 The polypeptide elongates from the N terminus 229
 Elongation continues and the polypeptide grows 229
 A release factor terminates translation 229

Regulation of Translation 230

Some antibiotics work by inhibiting translation 230
 Polysome formation increases the rate of protein synthesis 230

Posttranslational Events 231

Chemical signals in proteins direct them to their cellular destinations 231
 Many proteins are modified after translation 233

Mutations: Heritable Changes in Genes 233

Point mutations are changes in single bases 234

Chromosomal mutations are extensive changes in the genetic material 235

Mutations can be spontaneous or induced 236

Mutations are the raw material of evolution 236

13 The Genetics of Viruses and Prokaryotes 239**Using Prokaryotes and Viruses to Probe the Nature of Genes 239****Viruses: Reproduction and Recombination 240**

Scientists studied viruses before they could see them 240
 Viruses reproduce only with the help of living cells 240
 There are many kinds of viruses 241
 Bacteriophages reproduce by a lytic cycle or a lysogenic cycle 241
 Animal viruses have diverse reproductive cycles 242
 Many plant viruses spread with the help of vectors 243
 Viroids are infectious agents consisting entirely of RNA 244

Prokaryotes: Reproduction, Mutation, and Recombination 245

The reproduction of prokaryotes gives rise to clones 245
 Some bacteria conjugate, recombining their genes 245
 In transformation, cells pick up genes from their environment 247
 In transduction, viruses carry genes from one cell to another 247
 Plasmids are extra chromosomes in bacteria 248
 Transposable elements move genes among plasmids and chromosomes 249

Regulation of Gene Expression in Prokaryotes 249

Regulation of transcription conserves energy 250
 A single promoter controls the transcription of adjacent genes 250
 Operons are units of transcription in prokaryotes 251
 Operator-repressor control that induces transcription: The *lac* operon 251

Operator-repressor control that represses transcription: The *trp* operon 252

Protein synthesis can be controlled by increasing promoter efficiency 253

Control of Transcription in Viruses 254

Prokaryotic Genomes 255

Functional genomics relates gene sequences to functions 255

The sequencing of prokaryotic genomes has medical applications 256

What genes are required for cellular life? 256

14 The Eukaryotic Genome and Its Expression 259

The Eukaryotic Genome 259

The eukaryotic genome is larger and more complex than the prokaryotic genome 259

The yeast genome adds some eukaryotic functions onto a prokaryotic model 261

The nematode genome adds developmental complexity 262

The fruit fly genome has surprisingly few genes 262

Gene sequences for other organisms are rapidly becoming known 263

Repetitive Sequences in the Eukaryotic Genome 263

Highly repetitive sequences are present in large numbers of copies 263

Telomeres are repetitive sequences at the ends of chromosomes 263

Some moderately repetitive sequences are transcribed 264

Transposable elements move about the genome 264

The Structures of Protein-Coding Genes 265

Protein-coding genes contain non-coding internal and flanking sequences 265

Many eukaryotic genes are members of gene families 267

RNA Processing 268

The primary transcript of a protein-coding gene is modified at both ends 269

Splicing removes introns from the primary transcript 269

Transcriptional Control 270

Specific genes can be selectively transcribed 270

Genes can be inactivated by chromatin structure 273

A DNA sequence can move to a new location to activate transcription 275

Selective gene amplification results in more templates for transcription 275

Posttranscriptional Control 276

Different mRNA's can be made from the same gene by alternate splicing 276

The stability of mRNA can be regulated 276

Translational and

Posttranslational Control 276

The translation of mRNA can be controlled 277

The proteasome controls the longevity of proteins after translation 277

15 Cell Signaling and Communication 279

Signals 279

Cells receive signals from the physical environment and from other cells 280

Signaling involves a receptor, transduction, and effects 281

Receptors 282

Receptors have specific binding sites for their signals 282

There are several types of receptors 283

Transducers 285

Protein kinase cascades amplify a response to receptor binding 286

Cyclic AMP is a common second messenger 287

Two second messengers are derived from lipids 287

Calcium ions are involved in many transduction pathways 288

Nitric oxide is a gas that can act as a second messenger 289

Signal transduction is highly regulated 290

Effects 290

Membrane channels are opened 290

Enzyme activities are changed 291

Different genes are transcribed 291

Direct Intercellular

Communication 292

Animal cells communicate by gap junctions 292

Plant cells communicate by plasmodesmata 292

16 Development: Differential Gene Expression 294

The Processes of Development 294

Development consists of growth, differentiation, and morphogenesis 295

As development proceeds, cells become more and more specialized 295

Determination precedes differentiation 296

The Role of Differential Gene Expression in Cell Differentiation 296

Differentiation usually does not include an irreversible change in the genome 296

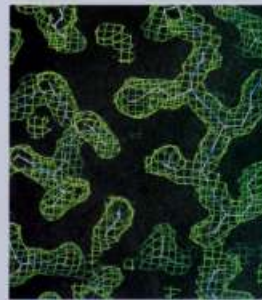
Stem cells can be induced to differentiate by environmental signals 299

Genes are differentially expressed in cell differentiation 299

The Role of Polarity in Cell Determination 300

The Role of Embryonic Induction in Cell Determination 301

Tissues direct the development of their neighbors by secreting inducers 301



- Single cells can induce changes in their neighbors 302
- The Role of Pattern Formation in Organ Development 302**
- Some cells are programmed to die 302
- Plants have organ identity genes 304
- Plants and animals use positional information 305
- The Role of Differential Gene Expression in Establishing Body Segmentation 305**
- Maternal effect genes determine polarity in *Drosophila* 305
- Segmentation and homeotic genes act after the maternal effect genes 306
- Drosophila* development results from a transcriptionally controlled cascade 307
- Homeotic mutations produce large-scale effects 307
- Homeobox-containing genes encode transcription factors 308
- Evolution and Development 308**

17 Recombinant DNA and Biotechnology 311

- Cleaving and Rejoining DNA 311**
- Restriction endonucleases cleave DNA at specific sequences 312
- Gel electrophoresis identifies the sizes of DNA fragments 312
- Recombinant DNA can be made in a test tube 313
- Cloning Genes 314**
- Genes can be inserted into prokaryotic or eukaryotic cells 315
- Vectors can carry new DNA into host cells 315
- There are many ways to insert recombinant DNA into host cells 316
- Genetic markers identify host cells that contain recombinant DNA 317
- Sources of Genes for Cloning 318**
- Gene libraries contain pieces of a genome 318
- A DNA copy of mRNA can be made 319
- DNA can be synthesized chemically in the laboratory 319
- DNA can be mutated in the laboratory 319

Some Additional Tools for DNA Manipulation 320

- Genes can be inactivated by homologous recombination 320
- DNA chips can reveal DNA mutations and RNA expression 321
- Antisense RNA and ribozymes can prevent the expression of specific genes 322
- Biotechnology: Applications of DNA Manipulation 322**
- Expression vectors can turn cells into protein factories 322
- Medically useful proteins can be made by DNA technology 323
- DNA manipulation is changing agriculture 325
- There is public concern about biotechnology 327
- DNA fingerprinting uses the polymerase chain reaction 328

18 Molecular Biology and Medicine 331

- Protein as Phenotype 332**
- Many genetic diseases result from abnormal or missing proteins 332
- Prion diseases are disorders of protein conformation 334
- Most diseases are caused by both heredity and environment 335
- Human genetic diseases have several patterns of inheritance 335
- Mutations and Human Diseases 336**
- The logical way to identify a gene is to start with its protein 336
- Chromosome deletions can lead to gene and then protein isolation 336
- DNA markers can point the way to important genes 337
- Human gene mutations come in many sizes 338
- Expanding triplet repeats demonstrate the fragility of some human genes 339
- Genomic imprinting shows that mammals need both a mother and father 339
- Detecting Human Genetic Variations 339**
- Screening for abnormal phenotypes can make use of protein expression 340

There are several ways to screen for abnormal genes 341

Cancer: A Disease of Genetic Changes 342

- Cancer cells differ from their normal counterparts 342
- Some cancers are caused by viruses 343
- Most cancers are caused by genetic mutations 343
- Two kinds of genes are changed in many cancers 344
- The pathway from normal cell to cancerous cell is complex 345
- Treating Genetic Diseases 346**
- One approach to treatment is to modify the phenotype 346
- Gene therapy offers the hope of specific treatments 347

Sequencing the Human Genome 348

- There are two approaches to genome sequencing 348
- The human genome project is more than just a sequence 350
- How should genetic information be used? 350

19 Natural Defenses against Disease 353

Defensive Cells and Proteins 354

- White blood cells play many defensive roles 354
- Immune system proteins bind pathogens or signal other cells 354

Innate Defenses 355

- Barriers and local agents defend the body against invaders 356
- Innate defenses include chemical and cellular processes 356

Specific Defenses: The Immune Response 358

- Four features characterize the immune response 358
- There are two interactive immune responses 359
- Clonal selection accounts for the characteristic features of the immune response 359
- Immunological memory and immunity result from clonal selection 360
- Animals distinguish self from nonself and tolerate their own antigens 360

B Cells: The Humoral Immune Response 362

Some B cells develop into plasma cells 362

Antibodies share a common structure, but may be of different classes 363

Hybridomas produce monoclonal antibodies 364

T Cells: The Cellular Immune Response 364

T cell receptors are found on two types of T cells 365

The major histocompatibility complex encodes proteins that present antigens to the immune system 366

Helper T cells and MHC II proteins contribute to the humoral immune response 367

Cytotoxic T cells and MHC I proteins contribute to the cellular immune response 367

MHC molecules underlie the tolerance of self 367

MHC molecules are responsible for transplant rejection 368

The Genetic Basis of Antibody Diversity 368

Antibody diversity results from DNA rearrangement and other mutations 369

How does a B cell produce a specific heavy chain? 370

The constant region is involved in class switching 371

The Evolution of Animal Defense Systems 372**Disorders of the Immune System 372**

An inappropriately active immune system can cause problems 372

AIDS is an immune deficiency disorder 373

HIV infection and replication occur in T_H cells 373

Treatments for HIV infection rely on knowledge of its molecular biology 374

Part Three EVOLUTIONARY PROCESSES



20 The History of Life on Earth 379

How Do We Know Earth Is Ancient? 380

Radioactivity provides a way to date rocks 380

How Has Earth Changed over Time? 382

The continents have changed position 382

Earth's climate has shifted between hot/humid and cold/dry conditions 383

Volcanoes have disrupted evolution 383

External events have triggered other changes on Earth 384

The Fossil Record 384

How complete is the fossil record? 384

The fossil record demonstrates several patterns 385

Life in the Remote Past 386

Diversity exploded during the Cambrian 387

Major changes continued during the Paleozoic era 387

Geographic differentiation increased during the Mesozoic era 389

The modern biota evolved during the Cenozoic era 390

Rates of Evolutionary Change 390

Evolutionary rates vary 391

Extinction rates vary over time 391

Patterns of Evolutionary Change 392

Three major faunas have dominated animal life on Earth 392

The size and complexity of organisms have increased 393

Predators have become more efficient 393

The Future of Evolution 393

21 The Mechanisms of Evolution 395

Charles Darwin and Adaptation 395

Darwin proposed a mechanism to explain adaptation and evolution 396

What have we learned about evolution since Darwin? 397

Genetic Variation within Populations 398

Fitness is the relative reproductive contribution of genotypes 398

Most populations are genetically variable 398

How do we measure genetic variation? 399

The Hardy-Weinberg Equilibrium 400

Why is the Hardy-Weinberg equilibrium important? 402

Microevolution: Changes in the Genetic Structure of Populations 402

Mutations are changes in genetic material 402

Migration of individuals followed by breeding produces gene flow 402

Random genetic drift may cause large changes in small populations 402

Nonrandom mating changes the frequency of homozygotes 404

Natural selection produces variable results 404

Studying Microevolution 406**Maintaining Genetic Variation 408**

Sexual reproduction amplifies existing genetic variation 408

Neutral genetic mutations accumulate within species 408

Much genetic variation is maintained in geographically distinct subpopulations 408

Frequency-dependent selection maintains genetic variation within populations 409

How Do Genotypes Determine Phenotypes? 410

Constraints on Evolution 410

Short-Term versus Long-Term Evolution 411

22 Species and Their Formation 413

What Are Species? 413

How Do New Species Arise? 414

Allopatric speciation requires total genetic isolation 414

Sympatric speciation occurs without physical separation 417

Parapatric speciation separates adjacent populations 418

Reproductive Isolating Mechanisms 418

Prezygotic barriers operate before mating 419

Postzygotic barriers operate after mating 419

Sometimes reproductive isolation is incomplete 419



Species may differ in relatively few genes 419

Variation in Speciation Rates 420

Species richness may favor speciation 420

Range size may affect speciation rates 421

Behavior may influence speciation rates 421

Environmental changes may trigger high speciation rates 421

Short generation times enhance speciation 421

Evolutionary Radiations 422

The Significance of Speciation 423

23 Reconstructing and Using Phylogenies 425

How Are Phylogenetic Trees Reconstructed? 425

Identifying ancestral traits 428

Reconstructing a simple phylogeny 428

Traits Used in Reconstructing Phylogenies 430

Morphology and development 430

Molecular traits 430

Phylogenetic Trees Have Many Uses 431

How often have traits evolved? 432

When did lineages split? 432

Why Classify Organisms? 432

The Hierarchical Classification of Species 433

Biological Classification and Evolutionary Relationships 435

Early classifications were non-evolutionary 435

Current biological classifications reflect evolutionary relationships 435

The Future of Systematics 437

24 Molecular and Genomic Evolution 438

What is Molecular Evolution? 438

Determining and Comparing the Structure of Macromolecules 440

Why do rates of nucleotide substitution vary so greatly? 441

Using biological molecules as molecular clocks 441

Where Do New Genes Come From? 442

Duplication of genes can lead to new gene families 442

Homologous genes may be found in distantly related organisms 444

How Do Proteins Acquire New Functions? 445

Gene families provide evidence of functional diversification 445

Lysozyme evolved a novel function 445

Genome Organization and Evolution 446

Using Biological Molecules to Reconstruct Phylogenetic Trees 447

Molecular Studies of Human Evolution 447

25 The Origin of Life on Earth 450

How Can We Study a Unique Event that Happened Several Billion Years Ago? 450

Necessary Conditions for the Origin of Life 451

Conditions on early Earth differed from those of today 451

Polymerization provided diverse macromolecules 452

Protobionts: Enclosing Prebiotic Systems 452

The evolution of membranes provided partial isolation 453

Membrane components became energy-transducing devices 453

RNA was probably the first biological catalyst 453

DNA evolved from an RNA template 454

Photosynthesis Is the Source of Atmospheric O₂ 454

Is Life Evolving from Nonlife Today? 455

Does Life Exist Elsewhere in the Universe? 455

Part Four THE EVOLUTION OF DIVERSITY



26 Bacteria and Archaea: The Prokaryotic Domains 459

Why Three Domains? 459

General Biology of the Prokaryotes 461

Prokaryotes and their associations
take a few characteristic forms 461

Prokaryotes lack nuclei, organ-
elles, and a cytoskeleton 462

Prokaryotes have distinctive
modes of locomotion 462

Prokaryotes have distinctive cell
walls 463

Prokaryotes reproduce asexually,
but genetic recombination does
occur 464

Prokaryotes have exploited many
metabolic possibilities 464

Prokaryotes in Their Environments 466

Prokaryotes are important players
in element cycling 466

Prokaryotes live on and in other
organisms 466

A small minority of bacteria are
pathogens 466

Prokaryote Phylogeny and Diversity 467

Nucleotide sequences of prokary-
otes reveal their evolutionary
relationships 467

Lateral gene transfer muddled the
phylogenetic waters 468

Mutations are the most important
source of prokaryotic variation
468

The Bacteria 468

Some bacteria are heat lovers 469

The Proteobacteria are a large and
diverse group 469

The Cyanobacteria are important
photoautotrophs 470

Spirochetes look like corkscrews
470

Chlamydias are extremely small
471

Most Firmicutes are Gram-positive
471

The Archaea 472

The Archaea share some unique
characteristics 472

Most Crenarchaeota live in hot,
acidic places 473

The Euryarchaeota live in many
amazing places 473

27 Protists and the Dawn of the Eukarya 476

Protists Defined 476

The Origin of the Eukaryotic Cell 476

The modern eukaryotic cell arose
in several steps 478

"Archaezoa": The little kingdom
that was 479

Many uncertainties remain 479

General Biology of the Protists 480

Protists have diverse means of
locomotion 480

Vesicles perform a variety of func-
tions 480

The cell surfaces of protists are
diverse 481

Many protists contain endosym-
bionts 481

Both asexual and sexual reproduc-
tion occur among the protists 482

Protist Diversity 483

Euglenozoa 483

Euglenoids have anterior flagella
483

Kinetoplastids have mitochondria
that edit their own RNA 483

Alveolata 484

Dinoflagellates are unicellular
marine organisms with two fla-
gella 484

Apicomplexans are parasites with
unusual spores 484

Ciliates have two types of nuclei
485

Stramenopila 487

Diatoms are everywhere in the
marine environment 487

The brown algae include the
largest protists 488

Many protist and all plant life
cycles feature alternation of gen-
erations 489

The oomycetes include water
molds and their relatives 490

Rhodophyta 491

Chlorophyta 492

Chlorophytes vary in shape and
cellular organization 492

Chlorophyte life cycles are diverse
492

There are green algae other than
chlorophytes 494

Choanoflagellida 494

A History of Endosymbiosis 494

Some Recurrent Body Forms 495

Amoebas form pseudopods 495

Actinopods have thin, stiff
pseudopods 496

Foraminiferans have created vast
limestone deposits 496

Slime molds release spores from
erect fruiting bodies 496

28 Plants without Seeds: From Sea to Land 500

The Plant Kingdom 500

There are twelve surviving phyla
of plants 501

Life cycles of plants feature alter-
nation of generations 501

The Plantae arose from a green
algal lineage 501

The Conquest of the Land 502

Adaptations to life on land distin-
guish plants from green algae 502

Most present-day plants have vas-
cular tissue 502

The Nontracheophytes:

**Liverworts, Hornworts, and
Mosses 503**

Nontracheophyte sporophytes are
dependent on gametophytes 504

Liverworts are the most ancient
surviving plant lineage 504

Hornworts evolved stomata as an
adaptation to terrestrial life 505

Water- and sugar-transport mecha-
nisms emerged in the mosses 506

Introducing the Tracheophytes 507

Tracheophytes have been evolving
for almost half a billion years 508

The earliest tracheophytes lacked roots and leaves 508
Early tracheophytes added new features 510

The Surviving Nonseed Tracheophytes 511

The club mosses are sister to the other tracheophytes 511
Horsetails grow at the bases of their segments 512
Present-day whisk ferns resemble the most ancient tracheophytes 512
Ferns evolved large, complex leaves 513
The sporophyte generation dominates the fern life cycle 513

29 The Evolution of Seed Plants 516

General Characteristics of the Seed Plants 516

The Gymnosperms: Naked Seeds 518

We know the early gymnosperms only as fossils 518
Conifers have cones but no motile cells 518

The Angiosperms: Flowering Plants 521

The sexual structures of angiosperms are flowers 521
Flower structure has evolved over time 522
Angiosperms have coevolved with animals 523
The angiosperm life cycle features double fertilization 524
Angiosperms produce fruits 525
Determining the oldest living angiosperm lineage 525
There are two large monophyletic groups of angiosperms 526
The origin of the angiosperms remains a mystery 526

30 Fungi: Recyclers, Killers, and Plant Partners 529

General Biology of the Fungi 529

Some fungi are unicellular 530
The body of a fungus is composed of hyphae 531
Fungi are in intimate contact with their environment 531

Fungi are absorptive heterotrophs 531

Most fungi reproduce both asexually and sexually 532

Many fungal life cycles include a dikaryon stage 533

Some fungi are pathogens 533

Diversity in the Kingdom Fungi 533

Chytrids probably resemble the ancestral fungi 533

Zygomycetes reproduce sexually by fusion of two gametangia 534

The sexual reproductive structure of ascomycetes is an ascus 535

The sexual reproductive structure of basidiomycetes is a basidium 538

Imperfect fungi lack a sexual stage 539

Fungal Associations 539

Mycorrhizae are essential to many plants 539

Lichens grow where no eukaryote has succeeded 540

31 Animal Origins and Lophotrochozoans 543

Descendants of a Common Ancestor 543

The Animal Way of Life 544

Clues to Evolutionary Relationships among Animals 544

Body Plans Are Basic Structural Designs 545

Sponges: Loosely Organized Animals 546

Cnidarians: Cell Layers and Blind Guts 549



Cnidarians are simple but specialized carnivores 549

Cnidarian life cycles 549

Ctenophores: Complete Guts and Tentacles 551

The Evolution of Bilaterally Symmetrical Animals 552

Protostomes and Deuterostomes: An Early Lineage Split 552

Simple Lophotrochozoans 553

Flatworms move by beating cilia 553

Rotifers are small but structurally complex 554

Lophophorates: An Ancient Body Plan 555

Bryozoans are colonial lophophorates 556

Brachiopods superficially resemble bivalve mollusks 557

Spiralians: Wormlike Body Plans 557

Segmented Bodies: Improved Locomotion 558

Annelids have many-segmented bodies 558

Mollusks lost segmentation but evolved shells 559

32 Ecdysozoans: The Molting Animals 564

Cuticles: Flexible, Unsegmented Exoskeletons 564

Some marine phyla have few species 565

Tough cuticles evolved in some unsegmented worms 566

The Arthropods and Their Relatives: Segmented External Skeletons 567

Related lineages had unjointed legs 568

Jointed legs appeared in the trilobites 569

Chelicerates Invaded the Land 570

Crustaceans: Diverse and Abundant 570

Uniramians are Primarily Terrestrial 571

Myriapods have many legs 571

Insects are the dominant uniramians 571

Themes in Protostome Evolution 574

33 Deuterostomate Animals 577

Deuterostomes and Protostomes: Shared Evolutionary Themes 577

Echinoderms: Complex Biradial Symmetry 579

Pelmatozoans have jointed arms 580

Eleutherozoans are the dominant echinoderms 581

Chordates: New Ways of Feeding 581

Acorn worms capture prey with a proboscis 581

The pharynx becomes a feeding device 582

A notochord appears in tunicates and lancelets 582

Origin of the Vertebrates 583

Jaws improve nutrition 584

Fins improve mobility 584

Swim bladders allow control of buoyancy 585

Colonizing the Land: Obtaining Oxygen from the Air 587

Amphibians invade the land 587

Amniotes colonize dry environments 588

Reptilian lineages diverge 589

Birds: Feathers and Flight 591

The Origin and Diversity of Mammals 593

Primates and the Origins of Humans 595

Human ancestors descended to the ground 597

Humans arose from australopithecine ancestors 597

Brains steadily became larger 598

Humans evolved language and culture 598

The human population has grown rapidly 600

Part Five THE BIOLOGY OF FLOWERING PLANTS



34 The Plant Body 603

Vegetative Organs of the Flowering Plant Body 603

Roots anchor the plant and take up water and minerals 605

Stems bear buds, leaves, and flowers 605

Leaves are the primary sites of photosynthesis 605

Plant Cells 607

Cell walls may be complex in structure 607

Parenchyma cells are alive when they perform their functions 608

Collenchyma cells provide flexible support while alive 608

Sclerenchyma cells provide rigid support after they die 608

Xylem transports water from roots to stems and leaves 608

Phloem translocates carbohydrates and other nutrients 608

Plant Tissues and Tissue Systems 610

Forming the Plant Body 611

Plants and animals develop differently 611

A hierarchy of meristems generates a plant's body 612

The root apical meristem gives rise to the root cap and the primary meristems 613

The products of the root's primary meristems become root tissues 613

The products of the stem's primary meristems become stem tissues 614

Many stems and roots undergo secondary growth 615

Leaf Anatomy Supports Photosynthesis 617

35 Transport in Plants 620

Uptake and Transport of Water and Minerals 620

Water moves through a membrane by osmosis 621

Uptake of mineral ions requires transport proteins 621

Water and ions pass to the xylem by way of the apoplast and symplast 622

Aquaporins control the rate, but not the direction, of water movement 624

Transport of Water and Minerals in the Xylem 624

Experiments ruled out some early models of transport in the xylem 624

Root pressure does not account for xylem transport 624

The transpiration-cohesion-tension mechanism accounts for xylem transport 625

A pressure bomb measures tension in the xylem sap 626

Transpiration and the Stomata 627

The guard cells control the size of the stomatal opening 627

Antitranspirants decrease water loss 628

Crassulacean acid metabolism correlates with an inverted stomatal cycle 628

Translocation of Substances in the Phloem 628

The pressure flow model appears to account for phloem translocation 630

Testing the pressure flow model 630

Plasmodesmata and material transfer between cells 632



36 Plant Nutrition 634

The Acquisition of Nutrients 634

- Autotrophs make their own organic compounds 635
- How does a stationary organism find nutrients? 635

Mineral Nutrients Essential to Plants 635

- Deficiency symptoms reveal inadequate nutrition 635
- Several essential elements fulfill multiple roles 636
- The identification of essential elements 637

Soils and Plants 637

- Soils are complex in structure 637
- Soils form through the weathering of rock 638
- Soils are the source of plant nutrition 638
- Fertilizers and lime are used in agriculture 639
- Plants affect soils 639

Nitrogen Fixation 640

- Nitrogen fixers make all other life possible 640
- Nitrogenase catalyzes nitrogen fixation 640
- Some plants and bacteria work together to fix nitrogen 641
- Biological nitrogen fixation does not always meet agricultural needs 641
- Plants and bacteria participate in the global nitrogen cycle 642

Sulfur Metabolism 643

Heterotrophic and Carnivorous Seed Plants 643

37 Plant Growth Regulation 646

Interacting Factors in Plant Development 646

- Several hormones and photoreceptors regulate plant growth 646
- Signal transduction pathways mediate hormone and photoreceptor action 647

From Seed to Death: An Overview of Plant Development 647

- The seed germinates and forms a growing seedling 647
- The plant flowers and sets fruit 648
- The plant senesces and dies 648

Ending Seed Dormancy and Beginning Germination 649

- Seed dormancy affords adaptive advantages 649
- Seed germination begins with the uptake of water 650
- The embryo must mobilize its reserves 650

Gibberellins: Regulators from Germination to Fruit Growth 650

- Foolish seedlings led to the discovery of the gibberellins 651
- The gibberellins have many effects 652

Auxin Affects Plant Growth and Form 652

- Plant movements led to the discovery of auxin 653
- Auxin transport is polar 653
- Auxin carrier proteins move auxin into and out of cells 654
- Light and gravity affect the direction of plant growth 654
- Auxin affects vegetative growth in several ways 655
- Auxin controls the development of some fruits 656
- Auxin promotes growth by acting on cell walls 656
- Plants contain specific auxin receptor proteins 657
- Auxin and other hormones evoke differentiation and organ formation 658

Cytokinins Are Active from Seed to Senescence 658

Ethylene: A Gaseous Hormone That Promotes Senescence 658

- Ethylene hastens the ripening of fruit 659

Ethylene affects stems in several ways 659

The ethylene signal transduction pathway is well understood 659

Abscissic Acid: The Stress Hormone 660

Hormones in Plant Defenses 660

Brassinosteroids: "New" Hormones with Multiple Effects 660

Light and Photoreceptors 661

- Phytochromes mediate the effects of red and far-red light 661
- Phytochromes have many effects 662
- There are multiple phytochromes 662
- Cryptochromes and phototropin are blue-light receptors 662

38 Reproduction in Flowering Plants 665

Many Ways to Reproduce 665

Sexual Reproduction 666

- The flower is an angiosperm's device for sexual reproduction 666
- Flowering plants have microscopic gametophytes 666
- Pollination enables fertilization in the absence of liquid water 667
- Some plants practice "mate selection" 668
- A pollen tube delivers male cells to the embryo sac 668
- Angiosperms perform double fertilization 668
- Embryos develop within seeds 668
- Some fruits assist in seed dispersal 670
- The transition to the flowering state 670
- Apical meristems can become inflorescence meristems 670
- A cascade of gene expression leads to flowering 671

Photoperiodic Control of Flowering 671

- There are short-day, long-day, and day-neutral plants 671
- The length of the night determines whether a plant will flower 672
- Circadian rhythms are maintained by a biological clock 673
- Is there a flowering hormone? 674

Vernalization and Flowering 675

Asexual Reproduction 676

There are many forms of asexual reproduction 676
Asexual reproduction is important in agriculture 677

39 Plant Responses to Environmental Challenges 679

Plant-Pathogen Interactions 679

Plants seal off infected parts to limit damage 679

Plants have potent chemical defenses against pathogens 680

The hypersensitive response is a localized containment strategy 680

Systemic acquired resistance is a form of long-term "immunity" 680

Some plant genes match up with pathogen genes 681

Plants and Herbivores: Benefits and Losses 681



Grazing increases the productivity of some plants 681

Some plants produce chemical defenses 682

Some secondary products play multiple roles 683

Many defenses depend on extensive signaling 683

Gene splicing may confer resistance to insects 684

Why don't plants poison themselves? 684

Part Six THE BIOLOGY OF ANIMALS



40 Physiology, Homeostasis, and Temperature Regulation 693

Homeostasis: Maintaining the Internal Environment 694

Tissues, Organs, and Organ Systems 695

Epithelial tissues cover the body and line organs 695

Connective tissues support and reinforce other tissues 696

Muscle tissues contract 697

Nervous tissues process information 697

Organs consist of multiple tissues 697

Physiological Regulation and Homeostasis 698

Set points and feedback information are required for regulation 698

Thermostats regulate temperature 699

Temperature and Life 699

Q_{10} is a measure of temperature sensitivity 700

An animal's sensitivity to temperature can change 700

Maintaining Optimal Body Temperature 700

Ectotherms and endotherms respond differently in metabolic chambers 701

Ectotherms and endotherms use behavior to regulate body temperature 702

The plant doesn't always win 685

Water Extremes: Dry Soils and Saturated Soils 685

Some plants evade drought 685

Some leaves have special adaptations to dry environments 685

Plants have other adaptations to a limited water supply 686

In water-saturated soils, oxygen is scarce 687

Too Much Salt: Saline Environments 688

Most halophytes accumulate salt 688

Halophytes and xerophytes have some similar adaptations 688

Habitats Laden with Heavy Metals 689

Hot and Cold Environments 689

Plants have ways of coping with high temperatures 690

Some plants are adapted to survival at low temperatures 690

Both ectotherms and endotherms control blood flow to the skin 702

Some ectotherms produce heat 703

Some fish elevate body temperature by conserving metabolic heat 704

Thermoregulation in Endotherms 705

Endotherms actively increase heat production or heat loss 705

Decreasing heat loss is important for life in the cold 706

Evaporation of water is an effective way to lose heat 707

The Vertebrate Thermostat 707

The vertebrate thermostat uses feedback information 707

Fever helps the body fight infections 709

Animals can save energy by turning down the thermostat 709

41 Animal Hormones 712

Hormones and Their Actions 712

Most hormones are distributed in the blood 713

Some hormones act locally 713
 Hormones do not evolve as rapidly as their functions 714
 Endocrine glands secrete hormones 714

Hormonal Control of Molting and Development in Insects 715

Hormones from the head control molting in insects 715
 Juvenile hormone controls development in insects 716

Vertebrate Endocrine Systems 717

The pituitary develops from outpocketings of the mouth and brain 717
 Negative feedback loops control hormone secretion 721
 Thyroxine controls cell metabolism 722
 Thyroid dysfunction causes goiter 722
 Calcitonin reduces blood calcium 723
 Parathormone elevates blood calcium 724
 Insulin and glucagon regulate blood glucose 724
 Somatostatin is a hormone of the brain and the gut 724
 The adrenal gland is two glands in one 724
 The sex steroids are produced by the gonads 726
 Changes in control of sex steroid production initiate puberty 276
 Melatonin is involved in biological rhythms and photoperiodicity 727
 The list of other hormones is long 727

Mechanisms of Hormone Action 728

The actions of hormones depend on receptors and signal transduction pathways 728
 Hormone receptors are either on the cell surface or in the cell interior 728
 Regulation of hormone receptors controls sensitivity of cells to hormones 729
 Responses to hormones can vary greatly 729



42 Animal Reproduction 732

Asexual Reproduction 732

Budding and regeneration produce new individuals by mitosis 733
 Parthenogenesis is the development of unfertilized eggs 733

Sexual Reproduction 734

Eggs and sperm form through gametogenesis 734
 A single body can function as both male and female 736
 Anatomical and behavioral adaptations bring eggs and sperm together 736
 The evolution of vertebrate reproductive systems parallels the move to land 737
 Reproductive systems are distinguished by where the embryo develops 738

The Human Reproductive System 739

Male sex organs produce and deliver semen 739
 Male sexual function is controlled by hormones 741
 Female sex organs produce eggs, receive sperm, and nurture the embryo 741
 The ovarian cycle produces a mature egg 742
 The uterine cycle prepares an environment for the fertilized egg 743
 Hormones control and coordinate the ovarian and uterine cycles 743

Human Sexual Behavior 745

Human sexual responses consist of four phases 745
 Humans use a variety of technologies to control fertility 745
 Reproductive technologies help solve problems of infertility 748

Sexual behavior transmits many disease organisms 750

43 Animal Development 752

Fertilization: Interactions of Sperm and Egg 753

Recognition molecules assure specificity in sperm-egg interactions 753
 Sperm entry triggers blocks to polyspermy and activates the egg 754
 The sperm and the egg make different contributions to the zygote 754
 Fertilization causes rearrangements of egg cytoplasm 755

Cleavage: Repackaging the Cytoplasm 756

The amount of yolk influences cleavage 757
 The orientation of mitotic spindles influences the pattern of cleavage 757
 Cleavage in mammals is unique 758
 Specific blastomeres generate specific tissues and organs 758

Gastrulation: Producing the Body Plan 759

Involution of the vegetal pole characterizes gastrulation in the sea urchin 759
 Gastrulation in the frog begins at the gray crescent 761
 The dorsal lip of the blastopore organizes formation of the embryo 762
 Reptilian and avian gastrulation is an adaptation to yolky eggs 763
 Mammals have no yolk, but retain the avian-reptilian gastrulation pattern 764

Neurulation: Initiating the Nervous System 765

The stage is set by the dorsal lip of the blastopore 765
 Body segmentation develops during neurulation 766

Extraembryonic Membranes 767

Four extraembryonic membranes form with contributions from all germ layers 767
 Extraembryonic membranes in mammals form the placenta 768

The extraembryonic membranes provide means of detecting genetic diseases 768

Human Pregnancy and Birth 769

Human pregnancy can be divided into three trimesters 769

Parturition is triggered by hormonal and mechanical stimuli 770

44 Neurons and Nervous Systems 773

Nervous Systems: Cells and Functions 773

Nervous systems process information 774

Neurons are the functional units of nervous systems 775

Glial cells are also important components of nervous systems 776

Neurons function in networks 776

Neurons: Generating and Conducting Nerve Impulses 776

Simple electrical concepts underlie neuronal functions 777

Ion pumps and channels generate resting and action potentials 777

Ion channels can alter membrane potential 779

Sudden changes in ion channels generate action potentials 780

Action potentials are conducted down axons without reduction in the signal 781

Ion channels and their properties can be studied directly 783

Action potentials can jump down axons 784

Neurons, Synapses, and Communication 785

The neuromuscular junction is a classic chemical synapse 785

The arrival of an action potential causes the release of neurotransmitter 786

The postsynaptic membrane integrates synaptic input 786

Synapses between neurons can be excitatory or inhibitory 786

The postsynaptic membrane sums excitatory and inhibitory input 787

There are two types of neurotransmitter receptors 788

Electrical synapses are fast but do not integrate information well 789

The action of a neurotransmitter depends on the receptor to which it binds 789

Glutamate receptors may be involved in learning and memory 789

To turn off responses, synapses must be cleared of neurotransmitter 791

Neurons in Networks 792

45 Sensory Systems 794

Sensory Cells, Sensory Organs, and Transduction 794

Sensation depends on which neurons in the CNS receive action potentials from sensory cells 794

Sensory organs are specialized for detecting specific stimuli 795

Sensory transduction involves changes in membrane potentials 795

Many receptors adapt to repeated stimulation 797

Chemoreceptors: Responding to Specific Molecules 797

Arthropods provide good examples for studying chemosensation 797

Olfaction is the sense of smell 797

Gustation is the sense of taste 798

Mechanoreceptors: Detecting Stimuli that Distort Membranes 799

Many different sensory cells respond to touch and pressure 799

Stretch receptors are found in muscles, tendons, and ligaments 801

Hair cells provide information about balance, orientation in space, and motion 801

Auditory systems use hair cells to sense sound waves 803

Photoreceptors and Visual Systems: Responding to Light 805

Rhodopsin is responsible for photosensitivity 805

Invertebrates have a variety of visual systems 807

Image-forming eyes evolved independently in vertebrates and cephalopods 807

The vertebrate retina receives and processes visual information 808

Sensory Worlds Beyond Our Experience 812

Some species can see infrared and ultraviolet light 812

Echolocation is sensing the world through reflected sound 812

Some fish can sense electric fields 812

46 The Mammalian Nervous System: Structure and Higher Functions 814

The Nervous System: Structure, Function, and Information Flow 814

A conceptual diagram of the nervous system traces information flow 815

The vertebrate CNS develops from the embryonic neural tube 816

Functional Subsystems of the Nervous System 817

The spinal cord receives and processes information from the body 817

The reticular system alerts the forebrain 818

The limbic system supports basic functions of the forebrain 818

Regions of the cerebrum interact to produce consciousness and control behavior 819

The cerebrum has increased in size and complexity 821



Information Processing by Neuronal Networks 821

- The autonomic nervous system controls organs and organ systems 821
- Neurons and circuits in the occipital cortex integrate visual information 823
- Cortical cells receive input from both eyes 824

Understanding Higher Brain Functions in Cellular Terms 824

- Sleeping and dreaming involve electrical patterns in the cerebrum 825
- Some learning and memory can be localized to specific brain areas 826
- Language abilities are localized in the left cerebral hemisphere 828
- What is consciousness? 829

47 Effectors: Making Animals Move 831**Cilia, Flagella, and Cell Movement 831**

- Cilia are tiny, hairlike appendages of cells 831
- Flagella are like long cilia 832
- Cilia and flagella are moved by microtubules 832
- Microtubules are intracellular effectors 833
- Microfilaments change cell shape and cause cell movements 833

Muscle Contraction 833

- Smooth muscle causes slow contractions of many internal organs 834
- Cardiac muscle causes the heart to beat 834
- Skeletal muscle causes behavior 835
- Actin-myosin interactions are controlled by Ca^{2+} 838
- Calmodulin mediates Ca^{2+} control of contraction in smooth muscle 839
- Single muscle twitches are summed into graded contractions 839
- Muscle fiber types determine endurance and strength 840

Skeletal Systems Provide Support for Muscles 841

- A hydrostatic skeleton consists of fluid in a muscular cavity 841
- Exoskeletons are rigid outer structures 842
- Vertebrate endoskeletons provide supports for muscles 843
- Bones develop from connective tissues 844
- Bones that have a common joint can work as a lever 845

Other Effectors 846

- Nematocysts capture prey and repel predators 846
- Chromatophores enable animals to change color 846
- Glands can be effectors 847
- Electric organs can be shocking 847

48 Gas Exchange in Animals 849**Respiratory Gas Exchange 849**

- Air is a better respiratory medium than water 850
- High temperatures create respiratory problems for aquatic animals 850
- Oxygen availability decreases with altitude 851
- Carbon dioxide is lost by diffusion 851
- Fick's law applies to all systems of gas exchange 852

Respiratory Adaptations for Gas Exchange 852

- Respiratory organs have large surface areas 852
- Ventilation and perfusion maximize partial pressure gradients 852

Mammalian Lungs and Gas Exchange 857

- Respiratory tract secretions aid breathing 857
- Lungs are ventilated by pressure changes in the thoracic cavity 858

Blood Transport of Respiratory Gases 860

- Hemoglobin combines reversibly with oxygen 860
- Myoglobin holds an oxygen reserve 861
- The affinity of hemoglobin for oxygen is variable 861
- Carbon dioxide is transported as bicarbonate ions in the blood 862

Regulating Breathing to Supply O_2 863

- Breathing is controlled in the brain stem 863
- Regulating breathing requires feedback information 864

49 Circulatory Systems 866**Circulatory Systems: Pumps, Vessels, and Blood 866**

- Some simple aquatic animals do not have circulatory systems 867
- Open circulatory systems move tissue fluid 867
- Closed circulatory systems circulate blood through tissues 867

Vertebrate Circulatory Systems 868

- Fishes have two-chambered hearts 868
- Amphibians have three-chambered hearts 869
- Reptiles have exquisite control of pulmonary and systemic circulation 869
- Birds and mammals have fully separated pulmonary and systemic circuits 870

The Human Heart: Two Pumps in One 871

- Blood flows from right heart to lungs to left heart to body 871
- The heartbeat originates in the cardiac muscle 872
- The EKG records the electrical activity of the heart 875

The Vascular System: Arteries, Capillaries, and Veins 875

- Arteries and arterioles have abundant elastic and muscle fibers 875
- Materials are exchanged between blood and tissue fluid in the capillaries 876
- Materials are exchanged in capillary beds by filtration, osmosis, and diffusion 876
- Lymphatic vessels return tissue fluid to the blood 877
- Blood flows back to the heart through veins 877
- Will you die of cardiovascular disease? 878

Blood: A Fluid Tissue 879



Red blood cells transport respiratory gases 879
Platelets are essential for blood clotting 880
Plasma is a complex solution 881

Control and Regulation of Circulation 882

Autoregulation matches local flow to local need 882
Arterial pressure is controlled and regulated by hormonal and neural mechanisms 882
Cardiovascular control in diving mammals conserves oxygen 884

50 Animal Nutrition 886

Nutrient Requirements 886

Energy can be measured in calories 887
Sources of energy can be stored in the body 888
Food provides carbon skeletons for biosynthesis 889
Animals need mineral elements in different amounts 891
Animals must obtain vitamins from food 891
Nutrient deficiency diseases 892

Adaptations for Feeding 893

The food of herbivores is often low in energy and hard to digest 893
Carnivores must detect, capture, and kill prey 893
Vertebrate species have distinctive teeth 894

Digestion 894

Tubular guts have an opening at each end 894

Digestive enzymes break down complex food molecules 896

Structure and Function of the Vertebrate Gut 896

Similar tissue layers are found in all regions of the vertebrate gut 896
Peristalsis moves food through the gut 897
Digestion begins in the mouth and the stomach 898
The small intestine is the major site of digestion 899
Nutrients are absorbed in the small intestine 900
Water and ions are absorbed in the large intestine 901
Herbivores have special adaptations to digest cellulose 902

Control and Regulation of Digestion 903

Autonomic reflexes coordinate functions in different regions of the gut 903
Hormones control many digestive functions 903

Control and Regulation of Fuel Metabolism 903

The liver directs the traffic of fuel molecules 904
Lipoproteins: The good, the bad, and the ugly 904
Fuel metabolism is controlled by hormones 904

The Regulation of Food Intake 906

Toxic Compounds in Food 906

Some toxins are retained and concentrated in organisms 907
Some toxins can bioaccumulate in the environment 907

The body cannot metabolize many synthetic toxins 907

51 Salt and Water Balance and Nitrogen Excretion 910

Tissue Fluids and Water Balance 910

Excretory organs control the solute potential of tissue fluid 911
Mechanisms used by excretory systems include filtration, secretion, and resorption 911

Distinguishing Environments and Animals in Terms of Salt and Water 911

Most marine invertebrates are osmoconformers 911
Osmoregulators regulate the concentration of their tissue fluids 911
The composition of tissue fluids can be regulated 912

Excreting Nitrogen 912

Aquatic animals excrete ammonia 912
Many terrestrial animals and some fishes excrete urea 912
Some terrestrial animals excrete uric acid 912
Most species produce more than one nitrogenous waste 913

The Diverse Excretory Systems of Invertebrates 914

Protonephridia excrete water and conserve salts 914
Metanephridia process coelomic fluid 914
Malpighian tubules are the excretory organs of insects 914

Vertebrate Excretory Systems Are Built of Nephrons 915

Blood is filtered in the glomerulus 916
The renal tubules convert glomerular filtrate to urine 916
Both marine and terrestrial vertebrates must conserve water 917

The Mammalian Excretory System 918

Kidneys produce urine, which the bladder stores 918
Nephrons have a regular arrangement in the kidney 919
Blood vessels also have a regular arrangement in the kidney 919

The volume of glomerular filtration is greater than the volume of urine 920

Most filtrate is resorbed by the proximal convoluted tubule 920

The loop of Henle creates a concentration gradient in the surrounding tissue 920

Urine is concentrated in the collecting ducts 920

Control and Regulation of Kidney Functions 921

The kidneys act to maintain the glomerular filtration rate 921

Blood pressure and osmolarity are regulated by ADH 922

The heart produces a hormone that influences kidney function 922

The kidneys help regulate acid-base balance 923

52 Animal Behavior 925

What, How, and Why Questions 926

Behavior Shaped by Inheritance 926

Deprivation and hybridization experiments test whether a behavior is inherited 927

Simple stimuli can trigger behaviors 928

Learning also shapes behavior 928

Imprinting is the learning of a complex releaser 929

Inheritance and learning interact to produce bird song 929

Genetically determined behavior is adaptive under certain conditions 930

Hormones and Behavior 931

Sex steroids determine the development and expression of sexual behavior in rats 931

Testosterone affects the development of the brain regions responsible for song in birds 932

The Genetics of Behavior 932

Hybridization experiments show whether a behavior is genetically determined 932

Artificial selection and crossbreeding experiments reveal the genetic complexity of behaviors 933

Molecular genetics techniques reveal specific genes that influence behavior 934

Communication 935

Chemical signals are durable but inflexible 935

Visual signals are rapid and versatile but are limited by directionality 935

Auditory signals communicate well over a distance 936

Tactile signals can communicate complex messages 936

Electric signals can also communicate messages 937

The Timing of Behavior: Biological Rhythms 937

Circadian rhythms control the daily cycle of behavior 937

Circannual rhythms control seasonal behaviors 940

Finding Their Way: Orientation and Navigation 940

Piloting animals orient themselves by means of landmarks 940

Homing animals can return repeatedly to a specific location 940

Migrating animals travel great distances with remarkable accuracy 941

Navigation is based on internal and environmental cues 941

Human Behavior 944

Part Seven ECOLOGY AND BIOGEOGRAPHY



53 Behavioral Ecology 947

Balancing Costs and Benefits of Behaviors 948

Choosing Where to Live and Forage 948

Features of an environment may indicate its suitability 948

How do animals choose what to eat? 949

Mating Tactics and Roles 950

Abundant sperm and scarce eggs drive mating behavior 950

Sexual selection often leads to exaggerated traits 950

Males attract mates in varied ways 951

Females are the choosier sex 952

Social and genetic partners may differ 952

Costs and Benefits of Social Behavior 952

Group living confers benefits and imposes costs 953

Categories of Social Acts 953

Altruism can evolve by means of natural selection 954

Unrelated individuals may behave altruistically toward one another 955

The Evolution of Animal Societies 956

Parents of many species care for their offspring 956

The environment influences the evolution of animal societies 957

54 Population Ecology 959

Population Structure: Patterns in Space and Time 959

Density is an important feature of populations 960

Spacing patterns reflect interactions among individuals 961

Age distributions reflect past events 961

Population Dynamics: Changes over Time 962

Births, deaths, and movements drive population dynamics 962
Life tables summarize patterns of births and deaths 962

Patterns of Population Growth 963

Population growth is influenced by the carrying capacity 964
Many species are divided into discrete subpopulations 965

Population Regulation 965

How does population density influence birth and death rates? 965
Disturbances affect population densities 966

Organisms cope with environmental changes by dispersing 966

Life Histories Influence Population Growth 967

Life histories include stages for growth, change in form, and dispersal 968

Life histories embody trade-offs 968

Offspring are like "money in the bank" 969

Can Humans Manage Populations? 970

Life history traits determine how heavily a population can be exploited 970

Life history information is used to control populations 970

Can we manage our own population? 972

55 Community Ecology 974

Types of Ecological Interactions 974

Resources and Consumers 975

Biotic interactions influence the conditions under which species can persist 975

Limiting resources determine the outcomes of interactions 975

Competition: Seeking and Using Scarce Resources 976

Competition can restrict species' ranges 976

Competition can reduce species' abundances 976

Predator-Prey and Parasite-Host Interactions 978

Parasite-host interactions 978

Predator-prey interactions 979

Predators may eliminate prey from some environments but not others 979

Predator-prey interactions change over evolutionary time 980

Neutral and Beneficial

Interspecific Interactions 982

In amensalism and commensalism, one participant is unaffected 983

Mutualisms benefit both participants 983

Coevolution of Interacting Species 985

Some Species Have Major Influences on Community Composition 986

Animals may change vegetation structure and species richness 986

Predators may change marine community structure 986

Temporal Changes in Communities 987

Indirect Effects of Interactions among Species 988

56 Ecosystems 991

Climates on Earth 991

Solar energy inputs drive global climates 992

Global atmospheric circulation influences climates 992

Global oceanic circulation is driven by winds 993

Energy Flow through Ecosystems 994

Photosynthesis drives energy flow in ecosystems 994

Energy flows through a series of organisms 995

Much energy is lost between trophic levels 996

Some ecosystems are not powered by direct sunlight 998

Humans manipulate ecosystem productivity 998

Cycles of Materials through Ecosystem Compartments 999

Oceans receive materials from the land and atmosphere 999



Lakes and rivers contain only a small fraction of Earth's water 999

The atmosphere regulates temperatures close to Earth's surface 1000

Land covers about one-fourth of Earth's surface 1001

Biogeochemical Cycles 1001

Water cycles through the oceans, fresh waters, atmosphere, and land 1001

Organisms profoundly influence the carbon cycle 1001

Few organisms can use elemental nitrogen 1003

Organisms drive the sulfur cycle 1004

The phosphorus cycle has no gaseous phase 1004

Humans have influenced biogeochemical cycles 1005

57 Biogeography 1007

Why Are Species Found Where They Are? 1007

Ancient events influence current distributions 1008

Modern biogeographic methods 1008

The Role of History in Biogeography 1008

Vicariance and dispersal can both explain distributions 1009

Biogeographers use parsimony to explain distributions 1010



Biogeographic histories are reconstructed from various kinds of evidence 1011

Earth can be divided into biogeographic regions 1011

Ecology and Biogeography 1011

The species richness of an area is determined by rates of colonization and extinction 1012

The island biogeographic model has been tested 1013

Species richness varies latitudinally 1014

Terrestrial Biomes 1014

Biomes are identified by their distinctive climates and dominant plants 1014

Pictures and graphs capture the essence of terrestrial biomes 1014

Tundra is found at high latitudes and in high mountains 1016

Boreal forests are dominated by evergreen trees 1017

Temperate deciduous forests change with the seasons 1018

Temperate grasslands are ubiquitous 1019

Cold deserts are high and dry 1020

Hot deserts form around 30° latitude 1021

The chaparral climate is dry and pleasant 1022

Thorn forests and savannas have similar climates 1023

Tropical deciduous forests occur in hot lowlands 1024

Tropical evergreen forests are rich in species 1025

Aquatic Biogeography 1026

Freshwater ecosystems have little water but many species 1026

Marine biogeographic regions are determined primarily by water temperature and nutrients 1026

Marine vicariant events influence species distributions 1027

Biogeography and Human History 1028

58 Conservation Biology 1030

Estimating Current Rates of Extinction 1030

Species-area relationships are used to estimate extinction rates 1030

Population models are used to estimate risks of extinction 1031

Why Do We Care about Species Extinctions? 1032

Determining Causes of Endangerment and Extinction 1033

Habitat destruction and fragmentation are important causes of extinction today 1034

Introduced pests, predators, and competitors have eliminated many species 1037

Overexploitation has driven many species to extinction 1038

Loss of mutualists threatens some species 1038

Global warming may cause species extinctions 1038

Preventing Species Extinctions 1039

Designing recovery plans 1039

Captive propagation has a role in conservation 1039

The cost of captive propagation is comparatively low 1040

Establishing Priorities for Conservation Efforts 1040

Where should parks be established? 1040

Some economic land uses are compatible with conservation 1041

Conservation requires large-scale planning 1041

Restoring Degraded Ecosystems 1041

Markets and Conservation 1042

Appendix: Some Measurements Used in Biology

Glossary

Illustration Credits

Index



At midnight on December 31, 1999, massive displays of fireworks exploded in many places on Earth as people celebrated a new millennium—the passage from one thousand-year time frame into the next—and the advent of the year 2000. One such millennial display took place above the Egyptian pyramids.

We are impressed with the size of the pyramids, how difficult it must have been to build them, and how ancient they are. The oldest of these awe-inspiring monuments to human achievement was built more than 4,000 years ago; in the human experience, this makes the Egyptian pyramids very, very old. Yet from the perspective of the age of Earth and the time over which life has been evolving, the pyramids are extremely young. Indeed, if the history of Earth is visualized as a 30-day month, recorded human history—the dawn of which coincides roughly with the construction of the earliest pyramids—is confined to the last 30 seconds of the final day of the month (Figure 1.1).

The development of modern biology depended on the recognition that an immense length of time was available for life to arise and evolve its current richness. But for most of human history, people had no reason to suspect that Earth was so old. Until the discovery of radioactive decay at the beginning of the twentieth century, no methods existed to date prehistoric events. By the middle of the nineteenth century, however, studies of rocks and the fossils they contained had convinced geologists that Earth was much older than had generally been believed. Darwin could not have conceived his theory of evolution by natural selection had he not understood that Earth was very ancient.

In this chapter we review the events leading to the acceptance of the fact that life on Earth has evolved over several billion years. We then summarize how evolutionary mechanisms adapt organisms to their environments, and we review the major milestones in the evolution of life on Earth. Finally, we briefly describe how scientists generate new knowledge, how they develop and test hypotheses, and how that knowledge can be used to inform public policy.

Organisms Have Changed over Billions of Years

Long before the mechanisms of biological evolution were understood, some people realized that organisms had changed over time and that living organisms had evolved from organisms no longer alive on Earth. In the 1760s, the French naturalist Count George-Louis Leclerc de Buffon (1707–1788) wrote his *Natural History of Animals*, which contained a clear statement of the possibility of evolution. Buffon originally believed that each species had been divinely created for a particular way of life, but as he studied animal anatomy, doubts arose. He observed that the limb bones of all mammals, no matter what their way of life, were re-

A Celebration of Time

One millennial fireworks display celebrating the year 2000 took place over the ancient pyramids of Egypt, structures that represent more than 4,000 years of human history but an infinitesimal portion of Earth's geologic history.



CHAPTER ONE

Each "day" represents about 150 million years.

Life appeared some time during "days" 3–4, or about 4 billion years ago.

U]



27

plants

First :and animals

28

Coal-forming 2.y forests

Insects

First mammals

Dinosaurs dominant

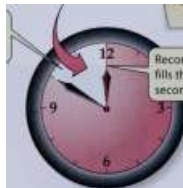
30

First birds

First flowering plants

Rise of mammals

Homo sapiens (modern humans) appeared in the last 10 minutes of day 30.



■ First hominids

Homo sapiens

1.1 Life's Calendar

If the history of Earth is depicted on the scale of a 30-day calendar month, recorded human history takes up only the last 30 seconds.

markably similar in many details (Figure 1.2). Buffon also noticed that the legs of certain mammals, such as pigs, have toes that never touch the ground and appear to be of no use. He found it difficult to explain the presence of these seemingly useless small toes by special creation.

Both of these troubling facts could be explained if mammals had not been specially created in their present forms, but had been modified over time from an ancestor that was common to all mammals. Buffon suggested that the limb bones of mammals might all be similar, and that the func-tionless toes of pigs might be inherited from ancestors with fully formed and functional toes. Button's idea was an early statement of evolution (descent with modification), although he did not attempt to explain how such changes took place.

Button's student Jean Baptiste de Lamarck (1744-1829) was the first person to propose a mechanism of evolutionary change. Lamarck suggested that lineages of organisms may change gradually over many generations as offspring inherit structures that have become larger and more highly developed as a result of continued use or, conversely, have become smaller and less developed as a result of disuse.

For example, Lamarck suggested that aquatic birds extend their toes while swimming, stretching the skin between them. This stretched condition, he thought, could be inherited by their offspring, which would in turn stretch their skin and pass this condition along to their offspring; birds with webbed feet would thereby evolve over a number of generations. Lamarck explained many other examples of adaptation in a similar way.

Today scientists do not believe that changes resulting from use and disuse can be inherited. But Lamarck did realize that species change with time. And after Lamarck, other naturalists and scientists speculated along similar lines.

By the middle of the nineteenth century, the climate of opinion among many scholars was receptive to a new theory of evolutionary processes. By then geologists had shown that Earth had existed and changed over millions of years, not merely a few thousand years. The presentation of a well-documented and thoroughly scientific argument for evolution then triggered a transformation of biology.

The theory of evolution by natural selection was proposed independently by Charles Darwin and Alfred Russel Wallace in 1858. We will discuss evolutionary theory in detail in Chapter 21, but its essential features are easy to understand. The theory rests on two facts and one inference drawn from them. The two facts are:

fills the last 30

seconds of day 30. ► The reproductive rates of all organisms,

even slowly reproducing ones, are sufficiently high that populations would quickly become enormous if mortality rates did not balance reproductive rates.

► Organisms of all types are variable, and offspring are similar to their parents because they inherit their features from them.

The inference is:

► The differences among individuals influence how well those individuals survive and reproduce. Traits that increase the probability that their bearers will survive and reproduce are more likely to be passed on to their offspring and to their offspring's offspring.

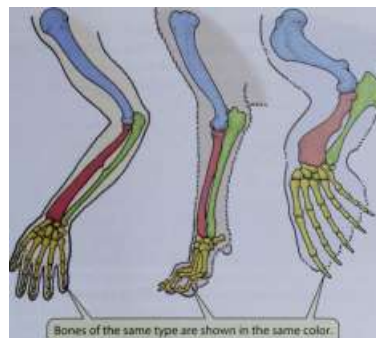
Darwin called the differential survival and reproductive success of individuals natural selection. The remarkable features of all organisms have evolved under the influence of natural selection. Indeed, the ability to evolve by means of natural selection clearly separates life from nonlife.

Biology began a major conceptual shift a little more than a century ago with the general acceptance of long-term evolutionary change and the recognition that differential survival and reproductive success is the primary process that adapts organisms to their environments. The shift has taken a long time because it required abandoning many components of an earlier worldview. The pre-Darwinian view held that the world was young, and that organisms had been created in their current forms. In the Darwinian view,

Human arm

Dog foreleg

Seal flipper



Bones of the same type are shown in the same color

1.2 Mammals Have Similar Limbs

Mammalian forelimbs have different purposes, but the number and types of their bones are similar, indicating that they have been modified overtime from a common ancestor.

the world is ancient, and both Earth and its inhabitants have been continually changing. In the Darwinian view of the world, organisms evolved their particular features because individuals with those features survived and reproduced better than individuals with different features.

Adopting this new view of the world means accepting not only the processes of evolution, but also the view that the living world is constantly evolving, and that evolutionary change occurs without any "goals." The idea that evolution is not directed toward a final goal or state has been more difficult for many people to accept than the process of evolution itself. But even though evolution has no goals, evolutionary processes have resulted in a series of profound changes—milestones—over the nearly 4 billion years life has existed on Earth.

Evolutionary Milestones

The following overview of the major milestones in the evolution of life provides both a framework for presenting the characteristics of life that will be described in this book and an overview of how those characteristics evolved during the

history of life on Earth.

Life arises from nonlife

All matter, living and nonliving, is made up of chemicals. The smallest chemical units are atoms, which bond together into molecules; the properties of those molecules are the subject of Chapter 2. The processes leading to life began nearly 4 billion years ago with interactions among small molecules that stored useful information.

The information stored in these simple molecules eventually resulted in the synthesis of larger molecules with

AN EVOLUTIONARY FRAMEWORK FOR BIOLOGY 3

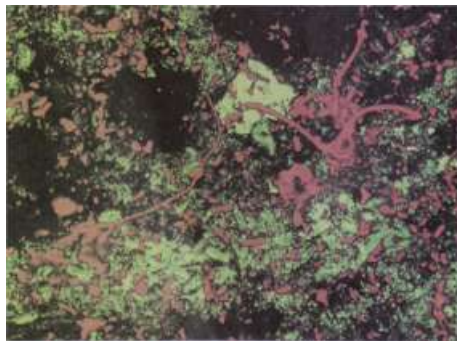
complex but relatively stable shapes. Because they were both complex and stable, these units could participate in increasing numbers and kinds of chemical reactions. Some of these large molecules—carbohydrates, lipids, proteins, and nucleic acids—are found in all living systems and perform similar functions. The properties of these complex molecules are the subject of Chapter 3.

Cells form from molecules

About 3.8 billion years ago, interacting systems of molecules came to be enclosed in compartments surrounded by membranes. Within these membrane-enclosed units, or cells, control was exerted over the entrance, retention, and exit of molecules, as well as the chemical reactions taking place within the cell. Cells and membranes are the subjects of Chapters 4 and 5.

Cells are so effective at capturing energy and replicating themselves—two fundamental characteristics of life—that since the time they evolved, they have been the unit on which all life has been built. Experiments by the French chemist and microbiologist Louis Pasteur and others during the nineteenth century convinced most scientists that, under present conditions on Earth, cells do not arise from noncellular material, but must come from other cells.

For 2 billion years, cells were tiny packages of molecules each enclosed in a single membrane. These prokaryotic cells lived autonomous lives, each separate from the other. They were confined to the oceans, where they were shielded from lethal ultraviolet sunlight. Some prokaryotes living today may be similar to these early cells (Figure 1.3).



1.3 Early Life May Have Resembled These Cells

"Rock-eating" bacteria, appearing red in this artificially colored micrograph, were discovered in pools of water trapped between layers of rock more than 1,000 meters below Earth's surface. Deriving chemical nutrients from the rocks and living in an environment devoid of oxygen, they may resemble some of the earliest prokaryotic cells.

CHAPTER ONE

To maintain themselves, to grow, and to reproduce, these early prokaryotes, like all cells that have subsequently evolved, obtained raw materials and energy from their environment, using these as building blocks to synthesize larger, carbon-containing molecules. The energy contained in these large molecules powered the chemical reactions necessary for the life of the cell. These conversions of matter and energy are called metabolism.

All organisms can be viewed as devices to capture, process, and convert matter and energy from one form to another; these conversions are the subjects of Chapters 6 and 7. A major theme in the evolution of life is the development of increasingly diverse ways of capturing external energy and using it to drive biologically useful reactions.

Photosynthesis changes Earth's environment

About 2.5 billion years ago, some organisms evolved the ability to use the energy of sunlight to power their metabolism. Although they still took raw materials from the environment, the energy they used to metabolize these materials came directly from the sun. Early photosynthetic cells were probably similar to present-day prokaryotes called cyanobacteria (Figure 1.4). The energy-capturing process they used—photosynthesis—is the basis of nearly all life on Earth today; it is explained in detail in Chapter 8. It used new metabolic reactions that exploited an abundant source of energy (sunlight), and generated a new waste product (oxygen) that radically changed Earth's atmosphere.

The ability to perform photosynthetic reactions probably accumulated gradually during the first billion years or so of evolution, but once this ability had evolved, its effects were dramatic. Photosynthetic prokaryotes became so abundant that they released vast quantities of oxygen gas (O_2) into the atmosphere. The presence of oxygen opened up new avenues of

evolution. Metabolic reactions that use O_2 , called aerobic metabolism, came to be used by most organisms on Earth. The oxygen in the air we breathe today would not exist without photosynthesis.

Over a much longer time, the vast quantities of oxygen liberated by photosynthesis had another effect. Formed from O_2 , ozone (O_3) began to accumulate in the upper atmosphere. The ozone slowly formed a dense layer that acted as a shield, intercepting much of the sun's deadly ultraviolet radiation. Eventually (although only within the last 800 million years of evolution), the presence of this shield allowed organisms to leave the protection of the oceans and establish new lifestyles on Earth's land surfaces.

Sex enhances adaptation

The earliest unicellular organisms reproduced by doubling their hereditary (genetic) material and then dividing it into two new cells, a process known as mitosis. The resulting progeny cells were identical to each other and to the parent. That is, they were clones. But sexual reproduction—the combining of genes from two cells in one cell—appeared

early during the evolution of life. Sexual reproduction is advantageous because an organism that combines its genetic information with information from another individual produces offspring that are more variable. Reproduction with variation is a major characteristic of life.

Variation allows organisms to adapt to a changing environment. Adaptation to environmental change is one of life's most distinctive features. An organism is adapted to a given environment when it possesses inherited features that enhance its survival and ability to reproduce in that environment. Because environments are constantly changing, organisms that produce variable offspring have an advantage over those that produce genetically identical "clones," because they are more likely to produce some offspring better adapted to the environment in which they find themselves.

Eukaryotes are "cells within cells"

As the ages passed, some prokaryotic cells became large enough to attack, engulf, and digest smaller cells, becoming the first predators. Usually the smaller cells were destroyed within the predators' cells. But some of these smaller cells survived and became permanently integrated into the operation of their hosts' cells. In this manner, cells with complex internal compartments arose. We call these cells eukaryotic cells. Their appearance slightly more than 1.5 billion years ago opened more new evolutionary opportunities.

Prokaryotic cells—the Bacteria and Archaea—have no membrane-enclosed compartments. Eukaryotic cells, on the



1.4 Oxygen Produced by Prokaryotes Changed Earth's Atmosphere

These modern cyanobacteria are probably very similar to early photosynthetic prokaryotes.

AN EVOLUTIONARY FRAMEWORK FOR BIOLOGY 5

7.5 Multiple Compartments Characterize Eukaryotic Cells

The nucleus and other specialized organelles probably evolved from small prokaryotes that were ingested by a larger prokaryotic cell. This is a photograph of a single-celled eukaryotic organism known as a protist.

other hand, are filled with membrane-enclosed compartments. In eukaryotic cells, genetic material—genes and chromosomes—became contained within a discrete nucleus and became increasingly complex. Other compartments became specialized for other purposes, such as photosynthesis. We refer to these specialized compartments as organelles (Figure 1.5).

Multicellularity permits specialization of cells

Until slightly more than 1 billion years ago, only single-celled organisms existed. Two key developments made the evolution of multicellular organisms—organisms consisting of more than one cell—possible. One was the ability of a cell to change its structure and functioning to meet the challenges of a changing environment. This was accomplished when prokaryotes evolved the ability to change from rapidly growing cells into resting cells called spores that could survive harsh environmental conditions. The second development allowed cells to stick together in a "clump" after they divided, forming a multicellular organism.

Once organisms could be composed of many cells, it became possible for the cells to specialize. Certain cells, for example,

could be specialized to perform photosynthesis. Other cells might become specialized to transport chemical materials such as oxygen from one part of an organism to another. Very early in the evolution of multicellular life, certain cells began to be specialized for sex—the passage of new genetic information from one generation to the next.

With the presence of specialized sex cells, genetic transmission became more complicated. Simple nuclear division—mitosis—was and is sufficient for the needs of most cells. But among the sex cells, or gametes, a whole new method of nuclear division—meiosis—evolved. Meiosis allows gametes to combine and rearrange the genetic infor-

mation from two distinct parent organisms into a genetic package that contains elements of both parent cells but is different from either. The recombinational possibilities generated by meiosis had great impact on variability and adaptation and on the speed at which evolution could occur.

Mitosis and meiosis are covered in detail in Chapter 9.

Controlling internal environments becomes more complicated

The pace of evolution, quickened by the emergence of sex and multicellular life, was also heightened by changes in Earth's atmosphere that allowed life to move out of the oceans and exploit environments on land. Photosynthetic green plants colonized the land, providing a rich source of energy for a vast array of organisms that consumed them. But whether it is made up of one cell or many, an organism must respond appropriately to its external environment. Life on land presented a new set of environmental challenges.

In any environment, external conditions can change rapidly and unpredictably in ways that are beyond an organism's control. An organism can remain healthy only if its internal environment remains within a given range of physical and chemical conditions. Organisms maintain relatively constant internal environments by making metabolic adjustments to changes in external and internal conditions such as temperature, the presence or absence of sunlight, the presence or absence of specific chemicals, the need for nutrients (food) and water, or the presence of foreign agents inside their bodies. Maintenance of a relatively stable internal condition—such as a constant human body temperature despite variation in the temperature of the surrounding environment—is called homeostasis. A major theme in the evolution of life is the development of increasingly complicated systems for maintaining homeostasis.

Multicellular organisms undergo regulated growth

Multicellular organisms cannot achieve their adult shapes or function effectively unless their growth is carefully regulated. Uncontrolled growth—one example of which is cancer—ultimately destroys life. A vital characteristic of living organisms is regulated growth. Achieving a functional multicellular organism requires a sequence of events leading from a single cell to a multicellular adult. This process is called development.

The adjustments that organisms make to maintain constant internal conditions are usually minor; they are not obvious, because nothing appears to change. However, at some time during their lives, many organisms respond to changing conditions not by maintaining their status, but by undergoing major cellular and molecular reorganization. An early form of such developmental reorganization was the prokaryotic spores that were generated in response to environmental stresses. A striking example that evolved much later is metamorphosis, seen in many modern in-

CHAPTER ONE



1.6 Organisms May Change Dramatically During Their Lives

The caterpillar, pupa, and adult are all stages in the life cycle of a monarch butterfly. The transition from one stage to another is triggered by internal signals.

sects, such as butterflies. In response to internal chemical signals, a caterpillar changes into a pupa and then into an adult butterfly (Figure 1.6).

The activation of gene-based information within cells and the exchange of signal information among cells produce the well-timed events that are required for the transition to the adult form. Genes control the metabolic processes necessary for life. The nature of the genetic material that controls these lifelong events has been understood only within the twentieth century;

it is the story to which much of Part Two of this book is devoted.

Altering the timing of development can produce striking changes. Just a few genes can control processes that result in dramatically different adult organisms. Chimpanzees and humans share more than 98 percent of their genes, but the differences between the two in form and in behavioral abilities—most notably speech—are dramatic (Figure 1.7). When we realize how little information it sometimes takes to create major transformations, the still mysterious process of speciation becomes a little less of a mystery.

Speciation produces the diversity of life

All organisms on Earth today are the descendants of a kind of unicellular organism that lived almost 4 billion years ago. The preceding pages described the major evolutionary events that have led to more complex living organisms. The course of this evolution has been accompanied by the storage of larger and larger quantities of information and increasingly complex mechanisms for using it. But if that were the entire story, only one kind of organism might exist



on Earth today. Instead, Earth is populated by many millions of kinds of organisms that do not interbreed with one another. We call these genetically independent groups of organisms species.

As long as individuals within a population mate at random and reproduce, structural and functional changes may occur, but only one species will exist. However, if a population becomes divided into two or more groups, and individuals can mate only with individuals in their own group, differences may accumulate with time, and the groups may evolve into different species.

The splitting of groups of organisms into separate species has resulted in the great variety of life found on Earth today, as described in Chapter 20. How species form is explained in Chapter 22. From a single ancestor, many species may arise as a result of the repeated splitting of populations. How biologists determine which species have descended from a particular ancestor is discussed in Chapter 23.



1.7 Genetically Similar Yet Very Different

1.7 Genetically Similar Yet Very Different

By looking at the two, you might be surprised to learn that chimpanzees and humans share more than 98 percent of their genes.



Sometimes humans refer to species as "primitive" or "advanced." These and similar terms, such as "lower" and "higher," are best avoided because they imply that some organisms function better than others. In this book, we use the terms "ancestral" and "derived" to distinguish characteristics that appeared earlier from those that appeared later in the evolution of life.

It is important to recognize that all living organisms are successfully adapted to their environments. The wings that allow a bird to fly and the structures that allow green plants to survive in environments where water is either scarce or overabundant are examples of the rich array of adaptations found among organisms (Figure 1.8).

The Hierarchy of Life

Biologists study life in two complementary ways:

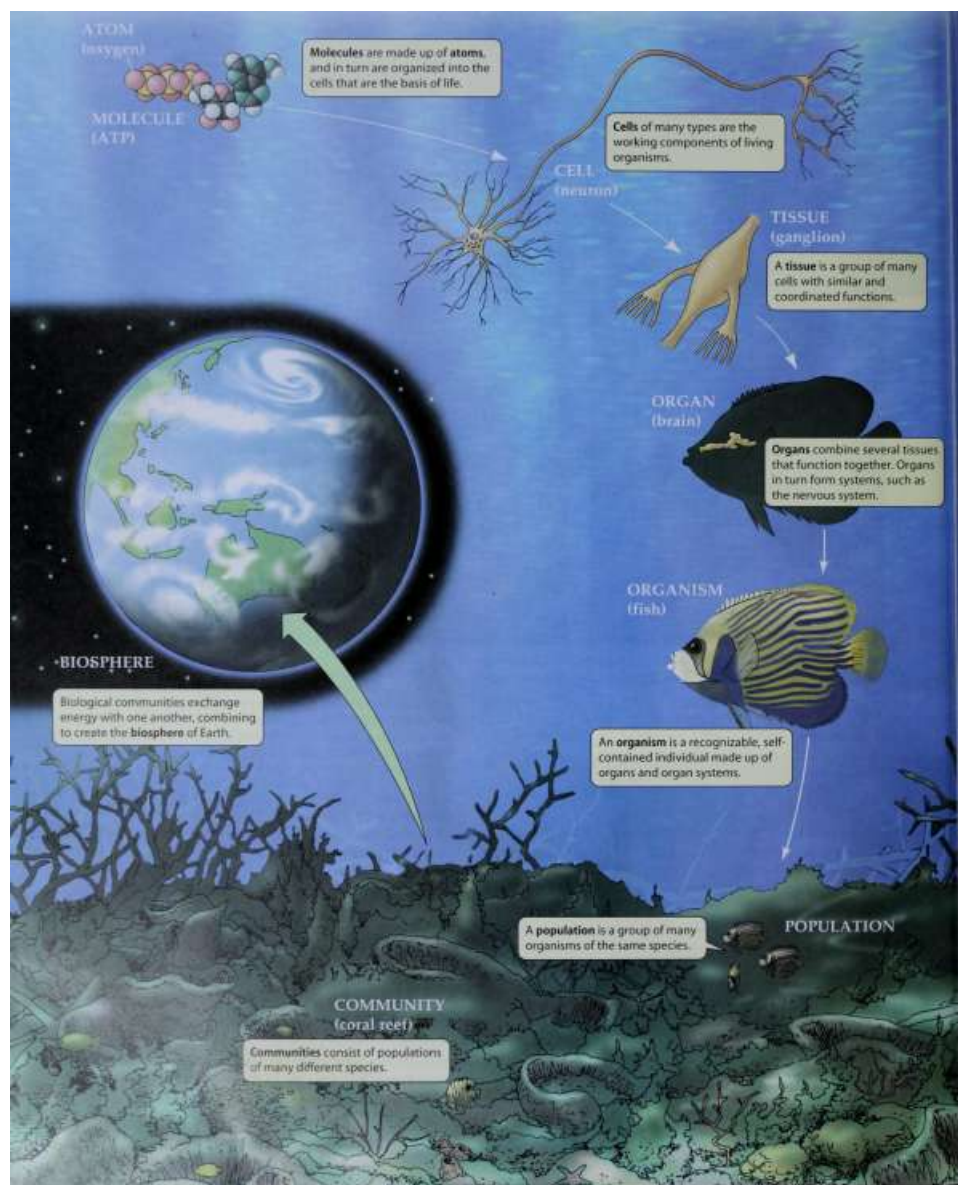
- They study structures and processes ranging from the simple to the complex and from the small to the large.

1.8 Adaptations to the Environment

(a) The long, pointed wings of the peregrine falcon allow it to accelerate rapidly as it dives on its prey, (fa) The action of a hummingbird's wings allows it to hover in front of a flower while it extracts nectar, (c) In a water-limited environment, this saguaro cactus stores water in its fleshy trunk. Its roots spread broadly to extract water immediately after it rains, (d) The aboveground root system of mangroves is an adaptation that allows these plants to thrive while inundated by salt water—an environment that would kill most terrestrial plants.

- They study the patterns of life's evolution over billions of years to determine how evolutionary processes have resulted in lineages of organisms that can be traced back to recent and distant ancestors.

These two themes of biological investigation help us synthesize the hierarchical relationships among organisms and the role of these relationships in space and time. We first describe the hierarchy of interactions among the units of biology from the smallest to the largest—from cells to the biosphere. Then we turn to the hierarchy of evolutionary relationships among organisms.



•4 1-9 The Hierarchy of Life

The individual organism is the central unit of study in biology, but understanding it requires a knowledge of many levels of biological organization both above and below it. At each higher level, additional and more complex properties and functions emerge.

Biologists study life at different levels

Biology can be visualized as a hierarchy in which the units, from the smallest to the largest, include atoms, molecules, cells, tissues, organs, organisms, populations, and communities (Figure 1.9).

The organism is the central unit of study in biology. Parts Five and Six of this book discuss organismal biology in detail. But to understand organisms, biologists must study life at all its levels of organization. Biologists study molecules, chemical reactions, and cells to understand the operations of tissues and organs. They study organs and organ systems to determine how organisms function and maintain internal homeostasis. At higher levels in the hierarchy, biologists study how organisms interact with one another to form social systems, populations, ecological communities, and biomes, which are the subjects of Part Seven of this book.

Each level of biological organization has properties, called emergent properties, that are not found at lower levels. For example, cells and multicellular organisms have characteristics and carry out processes that are not found in the molecules of which they are composed.

Emergent properties arise in two ways. First, many emergent properties of systems result from interactions among their parts. For example, at the organismal level, developmental interactions of cells result in a multicellular organism whose adult features are vastly richer than those of the single cell from which it grew. Other examples of properties that emerge through complex interactions are memory and emotions. In the human brain, these properties result from interactions among the brain's 10^{12} (trillion) cells with their 10^{15} (quadrillion) connections. No single cell, or even small group of cells, possesses them.

Second, emergent properties arise because aggregations have collective properties that their individual units lack. For example, individuals are born and they die; they have a life span. An individual does not have a birth rate or a death rate, but a population (composed of many individuals) does. Birth and death rates are emergent properties of a population. Evolution is an emergent property of populations that depends on variation in birth and death rates, which emerges from the different life spans and reproductive success of individuals in the various populations.

Emergent properties do not violate the principles that operate at lower levels of organization. However, emergent properties usually cannot be detected, predicted, or even suspected by studying lower levels. Biologists could never discover the existence of human emotions by studying sin-

AN EVOLUTIONARY FRAMEWORK FOR BIOLOGY 9

gle nerve cells, even though they may eventually be able to explain it in terms of interactions among many nerve cells.

Biological diversity is organized hierarchically

As many as 30 million species of organisms inhabit Earth today. Many times that number lived in the past but are now extinct. If we go back four billion years, to the origin of life, all organisms are believed to be descended from a single common ancestor. The concept of a common ancestor is crucial to modern methods of classifying organisms. Organisms are grouped in ways that attempt to define their evolutionary relationships, or how recently the different members of the group shared a common ancestor.

To determine evolutionary relationships, biologists assemble facts from a variety of sources. Fossils tell us where and when ancestral organisms lived and what they looked like. The physical structures different organisms share— toes among mammals, for example—can be an indication of how closely related they are. But a modern "revolution" in classification has emerged because technologies developed in the past 30 years now allow us to compare the genomes of organisms: We can actually determine how many genes different species share. The more genes species have in common, the more recently they probably shared a common ancestor.

Because no fossil evidence for the earliest forms of life remains, the decision to divide all living organisms into three major domains—the deepest divisions in the evolutionary history of life—is based primarily on molecular evidence (Figure 1.10). Although new evidence is constantly being brought to light, it seems clear that organisms belonging to a particular domain have been evolving separately from organisms in the other two domains for more than a billion years.

Organisms in the domains Archaea and Bacteria are prokaryotes—single cells that lack a nucleus and the other internal compartments found in the Eukarya. Archaea and Bacteria differ so fundamentally from each other in the chemical reactions by which they function and in the products they produce that they are believed to have separated into distinct evolutionary lineages very early during the evolution of life. These domains are covered in Chapter 26.

Members of the third domain have eukaryotic cells containing nuclei and complex cellular compartments called organelles. The Eukarya are divided into four groups—the protists and the classical kingdoms Plantae, Fungi, and Animalia (see Figure 1.10). Protists, the subject of Chapter 27, are mostly single-celled organisms. The remaining three kingdoms, whose members are all multicellular, are believed to have arisen from ancestral protists.

Some bacteria, some protists, and most members of the kingdom Plantae (plants) convert light energy to chemical energy by photosynthesis. The biological molecules that they produce are the primary food for nearly all other living organisms. The Plantae are covered in Chapters 28 and 29.

The Fungi, the subject of Chapter 30, include molds, mushrooms, yeasts, and other similar organisms, all of

10 CHAPTER ONE

Domains

Common ancestor of all organisms



BACTERIA

ARCHAEA

EUKARYA

Archaea and Eukarya share a common ancestor not shared by Bacteria.

7.70 The Major Groups of Organisms

The classification system used in this book divides Earth's organisms into three domains. The domain Eukarya contains numerous groups of unicellular and multicellular organisms. This "tree" diagram gives information on evolutionary relationships among the groups, as described in Chapter 23.

which are heterotrophs: They require a food source of energy-rich molecules synthesized by other organisms. Fungi absorb food substances from their surroundings and break them down (digest them) within their cells. They are important as decomposers of the dead bodies of other organisms.

Members of the kingdom Animalia (animals) are also heterotrophs. These organisms ingest their food source, digest the food outside their cells, and then absorb the products. Animals get their raw materials and energy by eating other forms of life. Perhaps because we are animals ourselves, we are often drawn to study members of this kingdom, which is covered in Chapters 31, 32, and 33.

The biological classification system used today has many hierarchical levels in addition to the ones shown in Figure 1.10. We will discuss the principal levels in Chapter 23. But to understand some of the terms we will use in the intervening chapters, you need to know that each species of organism is identified by two names. The first identifies the genus—a group of species that share a recent common ancestor—of which the species is a member. The second name is the species name. To avoid confusion, a particular combination of two names is assigned to only a single species. For example, the scientific name of the modern human species is *Homo sapiens*.

Asking and Answering "How?" and "Why?"

Because biology is an evolutionary science, biological

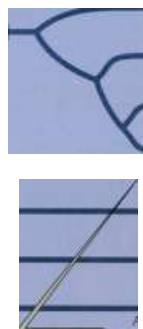
and products can be viewed from two different

but complementary perspectives. Biologists ask, and try to

answer, functional questions: How does it work? They also

Bacteria - R^+

Archaea



Protists

Plantae

Animalia

Modern protists are diverse and stem from several different lineages.

f

ask, and try to answer, adaptive questions: Why has it evolved to work that way?

Suppose, for example, that some marine biologists walking on mudflats in the Bay of Fundy, Nova Scotia, Canada, observe many amphipods (tiny relatives of shrimps and lobsters) crawling on the surface of the mud (Figure 1.11). Two obvious questions they might ask are

► How do these animals crawl?

► Why do they crawl?

To answer the "how" question, the scientists would investigate the molecular mechanisms underlying muscular contraction, nerve and muscle interactions, and the receipt of stimuli by the amphipods' brains. To answer the "why" question, they would attempt to determine why crawling on the mud is adaptive—that is, why it improves the survival and reproductive success of amphipods.

Is either of these two types of questions more basic or important than the other? Is any one of the answers more fundamental or more important than the other? Not really. The richness of possible answers to apparently simple questions makes biology a complex field, but also an exciting one. Whether we're talking about molecules bonding, cells dividing, blood flowing, amphipods crawling, or forests growing, we are constantly posing both how and why questions. To answer these questions, scientists generate hypotheses that can be tested.

Hypothesis testing guides scientific research

The most important motivator of most biologists is curiosity. People are fascinated by the richness and diversity of life, and they want to learn more about organisms and how they function and interact with one another. Curiosity is probably an adaptive trait. Humans who were motivated to learn about their surroundings are likely to have survived and reproduced better, on average, than their less curious relatives. We hope this book will help you share in the ex-



AN EVOLUTIONARY FRAMEWORK FOR BIOLOGY 1 1

7.7 7 An Amphipod from the Mud Flats

Scientists studied this tiny crustacean (whose actual size of approximately 1 centimeter is shown by the scale bar) in an attempt to see whether its behavior changes when it is infected by a parasitic worm. The female of this amphipod species is at the top; the lower specimen is a male.

citement biologists feel as they develop and test hypotheses. There are vast numbers of how and why questions for which we do not have answers, and new discoveries usually engender questions no one thought to ask before. Perhaps your curiosity will lead to an important new idea.

Underlying all scientific research is the hypothetico-deductive (H-D) approach by which scientists ask questions and test answers. The H-D approach allows scientists to modify and correct their beliefs as new observations and information become available. The method has five stages:

- ▶ Making observations.
- ▶ Asking questions.
- ▶ Forming hypotheses, or tentative answers to the questions.
- ▶ Making predictions based on the hypotheses.
- ▶ Testing the predictions by making additional observations or conducting experiments.

The data gained may support or contradict the predictions being tested. If the data support the hypothesis, it is subjected to still more predictions and tests. If they continue to support it, confidence in its correctness increases, and the hypothesis comes to be considered a theory. If the data do not support the hypothesis, it is abandoned or modified in accordance with the new information. Then new predictions are made, and more tests are conducted.

Applying the hypothetico-deductive method

The way in which marine biologists answered the question "Why do amphipods crawl on the surface of the mud rather than staying hidden within?" illustrates the H-D approach. As we saw above, the biologists observed something occurring in nature and formulated a question about it. To begin answering the question, they assembled available information on amphipods and the species that eat them.

They learned that during July and August of each year, thousands of sandpipers assemble for four to six weeks on the mudflats of the Bay of Fundy, during their southward migration from their Arctic breeding grounds to their wintering areas in South America (Figure 1.12). On these mud-

S*

U^ w

><~

i

3

i



J



7.72 Sandpipers Feed on Amphipods

Migrating sandpipers crowd the exposed tidal flats in search of food. By consuming infected amphipods, the sandpipers also become infected, serving as hosts and allowing the parasitic worm to complete its life cycle.

flats, which are exposed twice daily by the tides, they feed vigorously, putting on fat to fuel their next long flight. Amphipods living in the mud form about 85 percent of the diet of the sandpipers. Each bird may consume as many as 20,000 amphipods per day!

Previous observations had shown that a nematode (roundworm) parasitizes both the amphipods and the sandpipers. To complete its life cycle, the nematode must develop within both a sandpiper and an amphipod. The nematodes mature within the sandpipers' digestive tracts, mate, and release their eggs into the environment in the birds' feces. Small larvae hatch from the eggs and search for, find, and enter amphipods, where they grow through several larval stages. Sandpipers are reinfected when they eat parasitized amphipods.

GENERATING A HYPOTHESIS AND PREDICTIONS. Based on the

available information, biologists generated the following hypothesis: Nematodes alter the behavior of their amphipod hosts in a way that increases the chance that the worms will be

12 CHAPTER ONE



1.13 Collecting Field Data

Amphipods are collected from the mud to be tested for infection by parasites. Some of these crustaceans will be used in laboratory experiments.

passed on to sandpiper hosts. From this general hypothesis they generated two specific predictions.

► First, they predicted that amphipods infected by nematodes would increase their activity on the surface of the mud during daylight hours, when the sandpipers hunted by sight, but not at night, when the sandpipers fed less and captured prey by probing into the mud.

► Second, they predicted that only amphipods with late-stage nematode larvae—the only stage that can infect sandpipers—would have their behavior manipulated by the nematodes.

For each hypothesis proposing an effect, there is a corresponding null hypothesis, which asserts that the proposed effect is absent. For the hypothesis we have just stated, the null hypothesis is that nematodes have no influence on the behavior of their amphipod hosts. The alternative predictions that would support the null hypothesis are (1) that infected amphipods show no increase their activity either during the day or at night and (2) that all larval stages affect their hosts in the same

manner. It is important in hypothesis testing to generate and test as many alternate hypotheses and predictions as possible.

testing predictions. Investigators collected amphipods in the field, taking them from the surface and from within the mud, during the day and at night (Figure 1.13). They

found that during the day, amphipods crawling on the surface were much more likely to be infected with nematodes than were amphipods collected from within the mud. At night, however, there was no difference between the proportion of infected amphipods on the surface and those burrowing within the mud. This evidence supported the first prediction.

The field collections also showed that a higher proportion of the amphipods collected on the surface than of those collected from within the mud were parasitized by late-stage nematode larvae. However, amphipods crawling on the surface were no more likely to be infected by early-stage nematode larvae than were amphipods collected from the mud. These findings supported the second prediction.

To test the prediction that nematode larvae are more likely to affect amphipod behavior once they become infective, biologists performed laboratory experiments. They artificially infected amphipods with nematode eggs they obtained from sandpipers collected in the field. The infected amphipods established themselves in mud in laboratory containers.

By examining infected amphipods, investigators determined that it took about 13 days for the nematode larvae to reach the late, infective stage. By monitoring the behavior of the amphipods in the test tubes, the researchers determined that the amphipods were more likely to expose themselves on the surface of the mud once the parasites had reached the infective stage (Figure 1.14). This finding supported the second prediction.

Thus a combination of field and laboratory experiments, observation, and prior knowledge all supported the hypothesis that nematodes manipulate the behavior of their amphipod hosts in a way that decreases the survival of the amphipods, but increases the survival of the nematodes.

As is common practice in all the sciences, the researchers gathered all their data and collected them in a report, which they submitted to a scientific journal. Once such a report is published,* other scientists can evaluate the data, make their own observations, and formulate new ideas and experiments.

Experiments are powerful tools

The key feature of experimentation is the control of most factors so that the influence of a single factor can be seen clearly. In the laboratory experiments with amphipods, all individuals were raised under the same conditions. As a result, the nematodes reached the infective stage at about the same time in all of the infected amphipods.

Both laboratory and field experiments have their strengths and weaknesses. The advantage of working in a laboratory is that control of environmental factors is more

*In the case illustrated here, the data on amphipod behavior were published in the journal *Behavioral Ecology*, Volume 10, Number 4 (1998). D. McCurdy et al., "Evidence that the parasitic nematode *Skrjabinoclava* manipulates host *Corephium* behavior to increase transmission to the sandpiper, *Calidris pusilla*."

EXPERIMENT

Question: Is the manipulation of amphipod host behavior by a parasite equal at all times?

METHOD

Q Infect a known number of

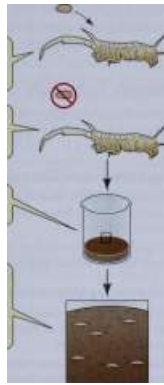
amphipods with nematode larvae (the experimental population).

I Prepare another population of amphipods known to be uninfected (the control population).

t

Place the two populations in mud-lined containers.

| Test and count amphipods of each population crawling on mud surface, once at day 3 and once at day 18. (Larvae reach the infective stage at day 13.)



RESULTS

I After 3 days, the proportions of the parasitized and unparasitized amphipod populations on the surface are similar.

3 days ^ later

18 days < later

T

Unparasitized controls

Parasitized (non-infective nematodes)

Unparasitized controls



Parasitized (infective nematodes)

100

Percent of amphipod population crawling on the mud surface during the day

I After the parasites become infective, the proportion of parasitized amphipods on the surface layer is much larger.

Conclusion: Parasitic nematodes influence their host's behavior only after they have reached the infective stage.

complete. Field experiments are more difficult because it is usually impossible to control more than a small number of environmental factors. But field experiments have one important advantage: Their results are more readily applicable to what happens where the organisms actually live and evolve. Just because an organism does something in the laboratory does not mean that it behaves the same way in nature. Because biologists usually wish to explain nature, not processes in the laboratory, combinations of laboratory

AN EVOLUTIONARY FRAMEWORK FOR BIOLOGY 13

7.74 An Experiment Demonstrates that Parasites Influence Amphipod Behavior

Amphipods are more likely to crawl on the surface of the mud, exposing themselves to being captured by sandpipers, when their parasitic nematodes have reached the stage at which they can infect a sandpiper.

and field experiments are needed to test most hypotheses about what organisms do.

A single piece of supporting evidence rarely leads to widespread acceptance of a hypothesis. Similarly, a single contrary result rarely leads to abandonment of a hypothesis. Results that do not support the hypothesis being tested can be obtained for many reasons, only one of which is that the hypothesis is wrong. Incorrect predictions may have been made from a correct hypothesis. A negative finding can also result from poor experimental design, or because an inappropriate organism was chosen for the test. For example, a species of sandpiper that fed only by probing in the mud for its prey would have been an unsuitable subject for testing the hypothesis that nematodes alter their hosts in a way to make them more visible to predators.

Accepted scientific theories are based on many kinds of evidence

A general textbook like this one presents hypotheses and theories that have been extensively tested, using a variety of methods, and are generally accepted. When possible, we illustrate hypotheses and theories with observations and experiments that support them, but we cannot, because of space constraints, detail all the evidence. Remember as you read that statements of biological "fact" are mixtures of observations, predictions, and interpretations.

No amount of observation could possibly substitute for experimentation. However, this does not mean that scientists are

insensitive to the welfare of the organisms with which they work. Most scientists who work with animals are continually alert to finding ways of getting answers that use the smallest number of experimental subjects and that cause the subjects the least pain and suffering.

Not all forms of inquiry are scientific

If you understand the methods of science, you can distinguish science from non-science. Recently some people have claimed that "creation science," sometimes called "scientific creationism," is a legitimate science that deserves to be taught in schools together with the evolutionary view of the world presented in this book. In spite of these claims, creation science is not science.

Science begins with observations and the formulation of hypotheses that can be tested and that will be rejected if significant contrary evidence is found. Creation science begins with the assertions, derived from religious texts, that Earth is only a few thousand years old and that all species of organisms were created in approximately their present forms. These assertions are not presented as a hypothesis

14 CHAPTER ONE

from which testable predictions can be derived. Advocates of creation science assume their assertions to be true and that no tests are needed, nor are they willing to accept any evidence that refutes them.

In this chapter we have outlined the hypotheses that Earth is about 4 billion years old, that today's living organisms evolved from single-celled ancestors, and that many organisms dramatically different from those we see today lived on Earth in the remote past. The rest of this book will provide evidence supporting this scenario. To reject this view of Earth's history, a person must reject not only evolutionary biology, but also modern geology, astronomy, chemistry, and physics. All of this extensive scientific evidence is rejected or misinterpreted by proponents of "creation science" in favor of their particular religious beliefs.

Evidence gathered by scientific procedures does not diminish the value of religious accounts of creation. Religious beliefs are based on faith—not on falsifiable hypotheses, as science is. They serve different purposes, giving meaning and spiritual guidance to human lives. They form the basis for establishing values—something science cannot do. The legitimacy and value of both religion and science is undermined when a religious belief is presented as scientific evidence.

Biology and Public Policy

During the Second World War and immediately thereafter, the physical sciences were highly influential in shaping public policy in the industrialized world. Since then, the biological sciences have assumed increasing importance. One reason is the discovery of the genetic code and the ability to manipulate the genetic constitution of organisms. These developments have opened vast new possibilities for improvements in the control of human diseases and agricultural productivity. At the same time, these capabilities have raised important ethical and policy issues. How much, and in what ways, should we tinker with the genetics of people and other species? Does it matter whether organisms are changed by traditional breeding experiments or by gene transfers? How safe are genetically modified organisms in the environment and in human foods?

Another reason for the importance of the biological sciences is the vastly increased human population. Our use of renewable and nonrenewable natural resources is stressing the ability of the environment to produce the goods and services upon which society depends. Human activities are causing the extinction of a large number of species and are resulting in the spread of new human diseases and the resurgence of old ones. Biological knowledge is vital for determining the causes of these changes and for devising wise policies to deal with them.

Therefore, biologists are increasingly called upon to advise governmental agencies concerning the laws, rules, and regulations by which society deals with the increasing number of problems and challenges that have at least a par-

tial biological basis. We will discuss these issues in many chapters of this book. You will see how the use of biological information can contribute to the establishment and implementation of wise public policies.

Chapter Summary

► If the history of Earth were a month with 30 days, recorded human history would occupy only the last 30 seconds. Review Figure 1.1

Organisms Have Changed over Billions of Years

► Evolution is the theme that unites all of biology. The idea of, and evidence for, evolution existed before Darwin. Review Figure 1.2

► The theory of evolution by natural selection rests on two simple observations and one inference from them.

Evolutionary Milestones

► Life arose from nonlife about 3.8 billion years ago when interacting systems of molecules became enclosed in membranes to form cells.

► All living organisms contain the same types of large molecules—carbohydrates, lipids, proteins, and nucleic acids.

► All organisms consist of cells, and all cells come from preexisting cells. Life no longer arises from nonlife.

► A major theme in the evolution of life is the development of increasingly diverse ways of capturing external energy and using it to drive biologically useful reactions.

► Photosynthetic single-celled organisms released large amounts of oxygen into Earth's atmosphere, making possible the oxygen-based metabolism of large cells and, eventually, multicellular organisms.

► Reproduction with variation is a major characteristic of life. The evolution of sexual reproduction enhanced the ability of organisms to adapt to changing environments.

^ Complex eukaryotic cells evolved when some large pro-karyotes engulfed smaller ones. Eukaryotic cells evolved the ability to "stick together" after they divided, forming multicellular organisms. The individual cells of multicellular organisms became modified for specific functions within the organism.

*■ A major theme in the evolution of life is the development of increasingly complicated systems for responding to changes in the internal and external environments and for maintaining homeostasis.

*■ Regulated growth is a vital characteristic of life. p- Speciation resulted in the millions of species living on Earth today.

>> Adaptation to environmental change is one of life's most distinctive features and is the result of evolution by natural selection.

The Hierarchy of Life

^ Biology is organized into a hierarchy of levels from molecules to the biosphere. Each level has emergent properties that are not found at lower levels. Review Figure 1.9 »- Species are classified into three domains: Archaea, Bacteria, and Eukarya. The domains Archaea and Bacteria consist of prokaryotic cells. The domain Eukarya contains the protists and the kingdoms Plantae, Fungi, and Animalia, all of which have eukaryotic cells. Review Figure 1.10

Asking and Answering "How?" and "Why?"

► Biologists ask two kinds of questions. "How" questions ask how organisms work. "Why" questions ask why they evolved to work that way.

► Both how and why questions are usually answered using a hypothetico-deductive (H-D) approach. Hypotheses are tentative answers to questions. Predictions are made on the basis of a hypothesis. The predictions are tested by observations and experiments, the results of which may support or refute the hypothesis. Review Figure 1.14

► Science is based on the formulation of testable hypotheses that can be rejected in light of contrary evidence. The acceptance on faith of already refuted, untested, or ^intestable assumptions is not science.

Biology and Public Policy

► Biologists are often called upon to advise governmental agencies on the solution of important problems that have a biological component.

For Discussion

1. According to the theory of evolution by natural selection, a species evolves certain features because they improve

5.

the chances that its members will survive and reproduce. There is no evidence, however, that evolutionary mechanisms have foresight or that organisms can anticipate future conditions. What, then, do biologists mean when they say, for example, that wings are "for flying"?

Why is it so important in science that we design and perform tests capable of rejecting a hypothesis?

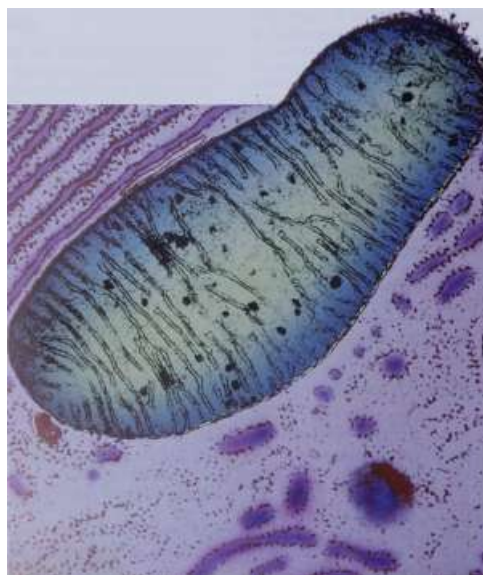
One hypothesis about the manipulation of a host's behavior by a parasite was discussed in this chapter, and some tests of that hypothesis were described. Suggest some other hypotheses about the ways in which parasites might change the behavior and physiology of their hosts. Develop some critical tests for one of these alternatives. What are the appropriate associated null hypotheses?

Some philosophers and scientists believe that it is impossible to prove any scientific hypothesis—that we can only fail to find a reason to reject it. Evaluate this view. Can you think of reasons why we can be more certain about rejecting a hypothesis than about accepting it?

Discuss one current environmental problem whose solution requires the use of biological knowledge. How well is biology being used? What factors prevent scientific data from playing a more important role in finding a solution to the problem?

Part One

The Cell



■

2

Small Molecules: Structure and Behavior



In recent years, some startling Discoveries have shown that life can exist in places we never dreamed possible. There are organisms living in hot springs at temperatures above the boiling point of water, beneath the frozen Antarctic ice, 2 miles below Earth's surface, 3 miles below the surface of the sea, in extremely acid environments, in extremely salty conditions, and even inside nuclear reactors. Such findings have rekindled interest in astrobiology, the science of and search for life outside Earth.

The one absolute requirement for life is water. Without water to act as a solvent for biochemicals, to receive wastes, to absorb heat, and to participate directly in chemical reactions, life would not exist as we know it. With strong recent evidence that there was once flowing water on Mars, and that Europa (one of Jupiter's moons) may have a thin crust of ice with liquid water below it, there is great excitement about the possibility of life on nearby extraterrestrial bodies.

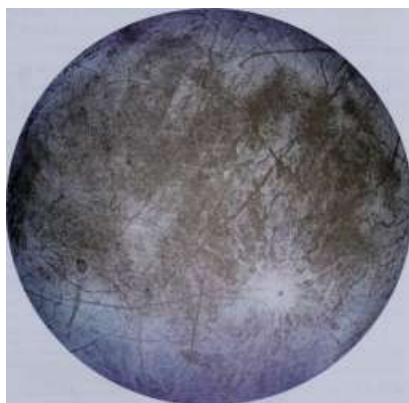
But what form would this life take? A major discovery of biology is that living things are composed of the same types of chemical elements as the vast nonliving portion of the universe. This mechanistic view—that life is chemically based and obeys universal physicochemical laws—is a relatively recent one in human history. The concept of a "vital force" responsible for life, different from the forces found in physics and chemistry, was common in Western culture until the nineteenth century, and many people still assume such a force exists. However, most scientists adhere to a mechanistic view of life.

Before describing how chemical elements are arranged in living creatures, we examine some fundamental chemical concepts. The first part of this chapter will address the constituents of matter: atoms. We examine their variety, their properties, and their capacity to combine with other atoms. Then we consider how matter changes. In addition to changes in state (solid to liquid to gas), substances undergo changes that transform both their composition and their characteristic properties. Then we return to a consideration of the structure and properties of water and its relationship to acids and bases. We close with a consideration of characteristic groups of atoms that contribute specific properties to larger molecules of which they are part, and which will be the subject of Chapter 3.

Atoms: The Constituents of Matter

More than a trillion (10^{12}) atoms could fit over the period at the end of this sentence. Each atom consists of a dense, positively charged nucleus, around which one or more negatively charged electrons move. The nucleus contains one or more protons and may contain one or more neutrons. Atoms and their component particles have mass, a property of all matter. Mass measures the quantity of matter present; the greater the mass, the greater the quantity of matter.

The mass of a proton serves as a standard unit of measure: the atomic mass unit (amu), or dalton (named after the English chemist John Dalton). A single proton or neutron has a mass of about 1 dalton, which is 1.7×10^{-24} grams ($0.0000000000000000000000017$ g). The mass of an electron is 9×10^{-28} g (0.0005 dalton). Because the mass of an electron is so much less than the mass of a proton or a neutron, the contribution of electrons to the mass of an atom can usually be ignored.



Life Off Earth?

Orbiting 400,000 miles above the giant planet Jupiter, Europa has a surface of water ice, possibly covering a slushy ocean. Where there is water, there could be, or could have been, life.

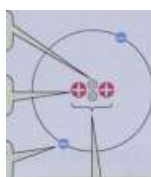
18 CHAPTER TWO

Even the smallest atoms, such as helium, have measurable mass:

Each neutron has a mass of 1 and no charge.

Each proton has a mass of 1 and a positive charge.

Each electron has negligible mass and negative charge.



The nucleus accounts for virtually all of the atom's mass, but occupies only 10^{-10} of its volume.

The positive electric charge of a proton is defined as a unit of charge. An electron has a negative charge equal and opposite to that of a proton. Thus the charge of a proton is + 1 unit, and that of an electron is -1 unit. Unlike charges (+/-) attract each other; like charges (+/+ or -/-) repel each other. The neutron, as its name suggests, is electrically neutral, so its charge is 0 unit. When the number of protons in an atom equals the number of electrons, the atom is electrically neutral. An atom with more or fewer electrons than protons has an electric charge and is called an ion; we will discuss ions in detail later in the chapter.

An element is made up of only one kind of atom

An element is a pure substance that contains only one type of atom. The element hydrogen consists only of hydrogen atoms; the element iron consists only of iron atoms. The atoms of each element have certain characteristics or properties that distinguish them from the atoms of other elements. The more than 100 elements found in the universe are arranged in the periodic table (Figure 2.1). These elements are not found in equal amounts. Earth's crust is half oxygen; 28% silicon; 8% aluminum; 3-5% each of sodium, magnesium, potassium, calcium, and iron; and much smaller amounts of the other elements.

About 98% of the mass of every living organism (bacterium, turnip, or human) is composed of just six elements: carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. Other elements are present in small amounts. The chemistry of the six major elements will be our primary concern here, but the others are not unimportant. Sodium and potassium, for example, are essential for nerves to function; calcium can act as a biological signal; iodine is a component of a vital hormone; and plants need molybdenum in order to incorporate nitrogen into biologically useful substances.

2. 1 The Periodic Table

The periodic table groups the elements according to their physical and chemical properties.



f

Masses in parentheses indicate unstable elements that decay rapidly to form other elements.

T

Elements without a chemical symbol are yet unnamed.

Lanthanide series

Actinide series

SMALL MOLECULES: STRUCTURE AND BEHAVIOR 19

The number of protons identifies the element

An element is distinguished from other elements by the number of protons in each of its atoms. This number, which does not change, is called the atomic number. An atom of hydrogen contains 1 proton, a helium atom has 2 protons, carbon has 6 protons, and plutonium has 94 protons. The atomic numbers of these elements are thus 1, 2, 6, and 94, respectively.

Every element except hydrogen has one or more neutrons in its nucleus. The mass number of an atom equals the total number of protons and neutrons in its nucleus. Because the mass of an electron is infinitesimal compared with that of a neutron or proton, electrons are ignored in calculating the mass number. The nucleus of a helium atom contains 2 protons and 2 neutrons; oxygen has 8 protons and 8 neutrons. Helium, therefore, has a mass number of 4 and oxygen a mass number of 16. The mass number may be thought of as the mass of the atom in daltons.

Each element has its own one- or two-letter chemical symbol. For example, H stands for hydrogen, He for helium, and O for oxygen. Some symbols come from other languages: Fe (from the Latin ferrum) stands for iron, Na (Latin natrium) for sodium, and W (German Wolfram) for tungsten. The periodic table (see Figure 2.1) gives the symbols for the 92 natural elements, as well as showing 26 elements (elements 93-118) that have been synthesized in laboratories but have not been found in nature.

In text, the atomic number and mass number of an element are written to the left of the element's symbol:

Proton

Neutron

Mass number ^A Atomic number Z

(J Symbol of element

Thus, hydrogen, carbon, and oxygen are written as ¹1H, ¹²6C, and ¹⁶8O, respectively.

Isotopes differ in number of neutrons

We have been speaking of elements as if each had only one atomic form, but this is not true. Isotopes of the same element all have the same number of protons, but differ in the number of neutrons in the atomic nucleus (Figure 2.2).

In nature, many elements exist as several isotopes. For example, the natural isotopes of carbon are ¹²6C, ¹³6C, and ¹⁴6C. Unlike the isotopes of hydrogen, which have special names (see Figure 2.2), the isotopes of most elements do not have distinct names. Rather, they are written in the form shown above and are referred to as carbon-12, carbon-13, and carbon-14, respectively. Most carbon atoms are ¹²6C, about 1.1 percent are ¹³6C, and a tiny fraction are ¹⁴6C. An element's atomic mass, or atomic weight,* is the average of the mass numbers of a representative sample of atoms of the element, with all isotopes in their normally occurring

Q Q



-H 3H

Isotopes of hydrogen

Hydrogen Deuterium Tritium

¹²6C ¹³6C ¹⁴6C

Isotopes of carbon

Carbon-12

1 proton 1 proton 1 proton 6 protons

1 neutron 2 neutrons 6 neutrons

Carbon-14

(-> protons 8 neutrons

2.2 Isotopes Have Different Numbers of Neutrons

Deuterium and tritium are rare isotopes of hydrogen. Unlike these two isotopes, isotopes of other elements do not have distinct names. Carbon-12 is the most common isotope of carbon; carbon-14 is a rare form.

proportions. The atomic weight of carbon is thus calculated to be 12.011.

Some isotopes, called radioisotopes, are unstable and spontaneously give off energy as a (alpha), (beta), or (gamma) radiation from the atomic nucleus. Such radioactive decay transforms the original atom into another atom, usually of another element. For example, carbon-14 loses a beta particle (actually an electron) to form nitrogen-14. Biologists and physicians can incorporate radioisotopes into molecules and use the emitted radiation as a tag to locate those molecules or to identify changes that the molecules undergo inside the body (Figure 2.3). Three radioisotopes commonly used in this way are ^3H (tritium), ^{14}C (carbon-14), and ^{32}P (phosphorus-32). In addition to these applications, radioisotopes can be used to date fossils (see Chapter 20).

Although radioisotopes are useful for experiments and in medicine, even low doses of their radiation have the potential to damage molecules and cells. Gamma radiation from cobalt-60 (^{60}Co) is used medically to damage or kill rapidly dividing cancer cells.

Electron behavior determines chemical bonding

When considering atoms, biologists are concerned primarily with electrons because the behavior of electrons explains how chemical changes occur in living cells. These changes, called chemical reactions or just reactions, are changes in the atomic composition of substances. The characteristic number of electrons in each atom of an element determines how its atoms react with other atoms. All chemical reactions involve changes in the relationships of electrons with one another.

The location of a given electron in an atom at any given time is impossible to determine. We can only describe a volume of space within the atom where the electron is likely to be. The region of space where the electron is found at least

The concepts of "weight" and "mass" are not identical. Weight is the measure of the Earth's gravitational attraction for mass; on another planet, the same quantity of mass would have a different weight. On Earth, however, the term "weight" is often used

as a measure of mass, and in biology one encounters the terms "weight" and "atomic weight" more frequently than "mass" and "atomic mass." Therefore, we will use "weight" for the remainder of this book.

20 CHAPTER TWO



2.3 A Radioisotope Used in Medicine

The thyroid gland takes up iodine and uses it in the synthesis of thyroid hormone. A patient suspected of having thyroid disease is injected with radioactive iodine, which allows the thyroid gland to be visualized by a scanning device.

Normal thyroid gland Enlarged thyroid gland

90 percent of the time is the electron's orbital (Figure 2.4). In an atom, a given orbital can be occupied by at most two electrons. Thus any atom larger than helium (atomic number 2) must have electrons in two or more orbitals. As Figure 2.4 shows, the different orbitals have characteristic forms and orientations in space.

The orbitals in turn constitute a series of electron shells, or energy levels, around the nucleus (Figure 2.5). The first, or innermost, electron shell consists of only one orbital, called an s orbital. Hydrogen (^1H) has one electron in its first shell; helium (^2He) has two. All other elements have two first-shell electrons, as well as electrons in other shells.

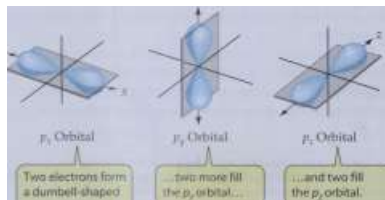
The second shell is made up of four orbitals (an s orbital and three p orbitals) and hence can hold up to eight electrons. The s orbitals fill with electrons first, and their electrons have the lowest energy. Subsequent shells have different numbers of orbitals, but the outermost shells usually hold only eight electrons.

In any atom, the outermost electron shell determines how the atom combines with other atoms; that is, how an atom behaves chemically. When an outermost shell consisting of four orbitals contains eight electrons, there are no unpaired electrons (see Figure 2.5). Such an atom is stable and will not react with other atoms. Examples of chemically inert elements are helium, neon, and argon.



The two electrons closest to the nucleus move in a spherical s orbital.

s Orbital



p x Orbital

___JL

Two electrons form a dumbbell-shaped x-axis (p_x) orbital...

...two more fill the y orbital...

...and two fill the z orbital.

S

The atoms of chemically reactive elements seek to attain the stable condition of having no unpaired electrons in their outer shells. They attain this stability by sharing electrons with other atoms, or by gaining or losing one or more electrons from their outermost shells. When they share electrons, atoms are bonded together. Such bonds create stable associations of atoms called molecules.

A molecule can be defined as two or more atoms linked by chemical bonds. The tendency of atoms in stable molecules to have eight electrons in their outermost shell is known as the octet rule. Many atoms in biologically important molecules—for example, carbon (C) and nitrogen (N)—follow the octet rule. However, some biologically important atoms are exceptions to the rule. Hydrogen (H) is an obvious exception, attaining stability when only two electrons occupy its single shell.

Chemical Bonds: Linking

How Many Atoms Together

A chemical bond is an attractive force that links two atoms to form a molecule. There are several kinds of chemical bonds (Table 2.1). In this section, we first discuss covalent bonds, the strong bonds that result from the sharing of electrons. Then we examine other kinds of interactions, including hydrogen bonds, that are weaker than covalent bonds but enormously important to biology. Finally, we consider ionic bonding, which results as a consequence of the loss or gain of electrons by atoms.

Covalent bonds consist of shared pairs of electrons

When two atoms attain stable electron numbers in their outer shells by sharing one or more pairs of electrons, a covalent bond is formed. Consider two hydrogen atoms

In close proximity, each with a single

unpaired electron in the outer shell. Each positively charged nucleus exerts some attraction on the other atom's un-

der-

der-

All p orbitals full

A

Six electrons fill all three p orbitals.

2.4 Electron Orbitals

Each orbital holds a maximum of two electrons. The s orbitals have a lower energy level and fill with electrons before the p orbitals do.



SMALL MOLECULES: STRUCTURE AND BEHAVIOR 21

First shell

Hydrogen (H)

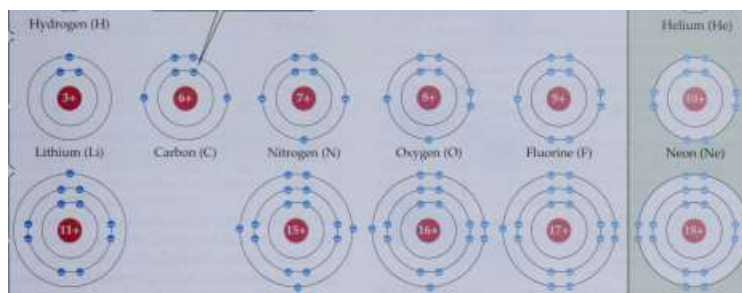
Nucleus

Electrons occupying the same orbital are shown as pairs.

Helium (He)

Second shell«

Third shell <



Sodium (Na)

Phosphorus (P)

Sulfur (S)

Chlorine (Cl)



Argon (Ar)

A

Elements whose outer shells contain unfilled or-bitals (unpaired electrons) are chemically reactive.

When all the orbitals in the outermost shell are filled, the element is not reactive (inert).

2.5 Electron Shells Determine the Reactivity of Atoms

Each orbital holds a maximum of two electrons, and each shell can hold a specific maximum number of electrons. Each shell must be filled before electrons move into the next shell. The energy level of electrons is higher in shells farther from the nucleus. An atom with unpaired electrons in its outermost shell may react (bond) with other atoms.

paired electron, but this attraction is balanced by each electron's attraction to its own nucleus. So the two unpaired electrons become shared by both atoms, filling the outer shells of both of them (Figure 2.6).

A carbon atom has a total of six electrons; two electrons fill its inner shell and four are in its outer shell. Because the outer shell can hold up to eight electrons, this atom can share electrons with up to four other atoms. Thus it can

Chemical Bonds and Interactions

NAME

BASIS OF INTERACTION

BOND ENERGY" (KCAL/MOL)

Covalent bond

Hydrogen bond

Ionic interaction

Sharing of electron pairs

Sharing of H atom

Attraction of opposite charges

50-110

3-7

3-7

van der Waals interaction Interaction of electron clouds

Hydrophobic interaction Interaction of nonpolar substances

H H H H

— C — C — H H — C — C —

H H H H

1-2

"Bond energy is the amount of energy needed to separate two bonded or interacting atoms under physiological conditions.

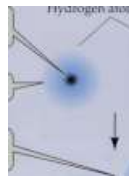
22 CHAPTER TWO

Each hydrogen atom has a nucleus with one proton...

Hydrogen atoms (2 H)

...and a shell with one electron.

Each electron is attracted to the other atom's nucleus...



...but the nucleus still attracts |_ its own electron. ["

I

If the atoms move closer they share the electron pair, linking them in a covalent bond and forming a hydrogen molecule.

Hydrogen molecule (H₂)

2.6 Electrons Are Shared in Covalent Bonds

Two hydrogen atoms combine to form a hydrogen molecule. Each electron is attracted to both protons. A covalent bond forms when the electron orbitals of the two atoms overlap.

form four covalent bonds. When an atom of carbon reacts with four hydrogen atoms, a substance called methane (CH₄) forms (Figure 2.7a and b). Thanks to electron sharing, the outer shell of methane's carbon atom is filled with eight electrons, and the outer shell of each hydrogen atom is also filled. Thus four covalent bonds—each consisting of a shared pair of electrons—hold methane together. Table 2.2 shows the covalent bonding capacities of some biologically significant elements.

orientation of covalent bonds. Covalent bonds are very strong. The thermal energy that biological molecules ordinarily have at body temperature is less than 1 percent of that needed to break covalent bonds. So biological molecules, most of which are put together with covalent bonds, are quite stable. A second property of covalent bonds is that, for a given pair of atoms, they are the same in length, angle, and direction, regardless of the larger molecule of which the particular bond is a part. The four filled orbitals around the carbon nucleus of methane, for example, distribute themselves in space so that the bonded hydrogens are directed to the corners of a regular tetrahedron with carbon in the center (Figure 2.7c). This three-dimensional structure of carbon and hydrogen is the same in complicat-

2.7 Covalent Bonding with Carbon

Differs notations of covalent bond formation in methane

(CH₄). (a) Illustrating the filling and stabilizing of the

outer electron shells in carbon and hydrogen atoms, (b) Two common ways of representing bonds, (c) The spatial orientation of methane's bonds, represented in two ways.

1

2.2

Covalent Bonding Capabilities of Some Biologically Important Elements

element

USUAL NUMBER OF COVALENT BONDS

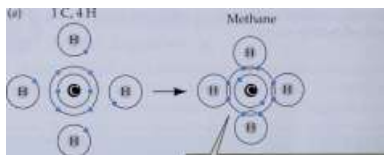
Hydrogen (H) Oxygen (O) Sulfur (S) Nitrogen (N) Carbon (C) Phosphorus (P)

ed proteins as it is in the simple methane molecule. It makes the prediction of biological structure possible.

Although the orientation of orbitals and the shapes of molecules differ depending on the kinds of atoms involved and how they are linked together, it is essential to remember that all molecules occupy space and have three-dimensional shapes. The shapes of molecules contribute to their biological functions, as we will see in Chapter 3.

multiple covalent bonds. A covalent bond is represented by a line between the chemical symbols for the atoms. A bond in which a single pair of electrons is shared is called a

Methane



Carbon can complete its outer shell by sharing the electrons of four hydrogen atoms, forming methane.

(b) 1 C, 4 H H H

Methane

H

H

H

H

H:C:H or H—C—H

ll

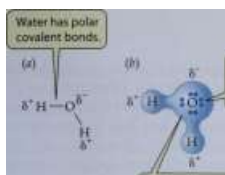
There are four electrons in the unfilled outer orbitals of carbon.

Each line or pair of dots represents a shared pair of electrons.



Hydrogens form corners of a regular tetrahedron.

This space-filling model shows the shape methane presents to its environment.



Unshared pairs of electrons are not part of the covalent bond.

Water's bonding electrons are shared unequally; electron density is greatest around the oxygen atom.

2.8 The Polar Covalent Bond in the Water Molecule

(a) A covalent bond between atoms with different electronegativities is a polar covalent bond, and has partial (δ) charges at the ends, (b) In water, the electrons are displaced toward the oxygen atom and away from the hydrogen atoms.

single bond (for example, H-H, C-H). When four electrons (two pairs) are shared, the link is called a double bond (C=C). In the gas ethylene ($\text{H}_2\text{C}=\text{CH}_2$), two carbon atoms share two pairs of electrons. Triple bonds (six shared electrons) are rare, but there is one in nitrogen gas ($\text{N}=\text{N}$), the chief component of the air we breathe. In the covalent bonds in these five examples, the electrons are shared more or less equally between the nuclei; consequently, all regions of the bonds are identical.

unequal sharing of electrons. If two atoms of the same element are covalently bonded, there is an equal sharing of the pair(s) of electrons in the outer shell. However, when the two atoms are of different elements, the sharing is not necessarily equal. One nucleus may exert a greater attractive force on the electron pair than the other nucleus, and so the pair tends to be closer to that atom.

The attractive force that an atom exerts on electrons is its electronegativity. It depends on how many positive charges a nucleus has (nuclei with more protons are more positive and thus more attractive to electrons) and how far away the electrons are from the nucleus (closer means more electronegativity). The closer two atoms are in electronegativity, the more equal their sharing of electrons will be.

Table 2.3 shows the electronegativities of some elements important in biological systems. Looking at the table, it is

SMALL MOLECULES: STRUCTURE AND BEHAVIOR 23

obvious that two oxygen atoms, both with electronegativity of 3.5, will share electrons equally in a covalent bond. So will two hydrogen atoms (both with 2.1). But when hydrogen bonds with oxygen to form water, the electrons involved are unequally shared: They tend to be nearer to the oxygen nucleus because it is the more electronegative of the two. The result is called a polar covalent bond (Figure 2.8).

Because of this unequal sharing of electrons, the oxygen end of the hydrogen-oxygen bond has a slightly negative charge (symbolized δ^- and spoken as "delta negative," meaning a partial unit of charge), and the hydrogen end is slightly positive (δ^+). The bond is polar because these opposite charges are separated at the two ends of the bond. The partial charges that result from polar covalent bonds produce polar molecules or polar regions of large molecules. Polar bonds greatly influence the interactions between molecules that contain them.

Hydrogen bonds may form between molecules

In liquid water, the negatively charged oxygen (δ^-) atom of one water molecule is attracted to the positively charged hydrogen (δ^+) atoms of another water molecule. (Remember, negative charges attract positive charges.) The bond resulting from this attraction is called a hydrogen bond.

Hydrogen bonds are not restricted to water molecules. They may form between an electronegative atom and a hydrogen covalently bonded to a different electronegative atom (Figure 2.9).

A hydrogen bond is a weak bond; it has about one-tenth (10%) of the strength of a covalent bond between a hydrogen atom and an oxygen atom (see Table 2.1). However, where many hydrogen bonds form, they have considerable strength and greatly influence the structure and properties of substances. Later in this chapter we'll see how hydrogen bonding in water contributes to many of the properties that make water significant for living systems. Hydrogen bonds also play important roles in determining and maintaining the three-dimensional shapes of giant molecules such as DNA and proteins (see Chapter 3).

8 4 H

8 + H

Polar

- covalent -

bond

O .

' N^J The hydrogen > bond is a weak attraction...

h -:o:

5 + ,

Two water molecules



...shared between two electronegative atoms.

Two parts of one large molecule (or two large molecules)

2.9 Hydrogen Bonds Can Form Between or within Molecules

Hydrogen bonds can form between two molecules or, if a molecule is large, between two different parts of the same molecule. Covalent and polar covalent bonds, on the other hand, are always found within molecules.

24 CHAPTER TWO

Ions form bonds by electrical attraction

When one interacting atom is much more electronegative than the other, a complete transfer of one or more electrons may

take place. Consider sodium (electronegativity 0.9) and chlorine (3.1). A sodium atom has only one electron in its outermost shell; this condition is unstable. A chlorine atom has seven electrons in its outer shell—another unstable condition. Since the electronegativities of these elements are so different, any electrons involved in bonding will tend to be much nearer to the chlorine nucleus—so near, in fact, that there is a complete transfer of the electron from one element to the other (Figure 2.10). This reaction between sodium and chlorine makes both atoms more stable. The result is two ions. Ions are electrically charged particles that form when atoms gain or lose one or more electrons.

► The sodium ion (Na^+) has a +1 unit charge because it has one less electron than it has protons. The outermost electron shell of the sodium ion is full, with eight electrons, so the ion is stable. Positively charged ions are called cations.

► The chloride ion (Cl^-) has a -1 unit charge because it has one more electron than it has protons. This additional electron gives Cl^- an outer shell with a stable load of eight electrons. Negatively charged ions are called anions.

Chlorine "steals" an electron from sodium.



Sodium atom (Na)

(11 protons, 11 electrons)

Chlorine atom (Cl)

(17 protons, 17 electrons)

\

The atoms are now electrically charged ions.



Sodium ion (Na^+)

(11 protons, 10 electrons)

Chloride ion (Cl^-)

(17 protons, 18 electrons)

After the transfer of the electron, both ions have full electron shells and are thus stable.

2.10 Formation of Sodium and Chloride Ions

If a sodium atom reacts with a chlorine atom, the more electronegative chlorine acquires a more stable, filled outer shell by obtaining an electron from the sodium. In so doing, the chlorine atom becomes a negatively charged chloride ion (Cl^-). The sodium atom, upon losing the electron, becomes a positively charged sodium ion (Na^+).

Some elements form ions with multiple charges by losing or gaining more than one electron. Examples are Ca^{2+} (calcium ion, created from a calcium atom that has lost two electrons) and Mg^{2+} (magnesium ion). Two biologically important elements each yield more than one stable ion: Iron yields Fe^{2+} (ferrous ion) and Fe^{3+} (ferric ion), and copper yields Cu^+ (cuprous ion) and Cu^{2+} (cupric ion). Groups of covalently bonded atoms that carry an electric charge are called complex ions; examples include NH_4^+ (ammonium ion), SO_4^{2-} (sulfate ion), and PO_4^{3-} (phosphate ion).

The charge from an ion radiates from it in all directions. Once they form, ions are usually stable, and no more electrons are lost or gained. Ions can form stable bonds, resulting in stable solid compounds such as sodium chloride (NaCl) and potassium phosphate (K_3PO_4).

Ionic bonds are bonds formed by electrical attractions between ions bearing opposite charges. In sodium chloride—familiar to us as table salt—cations and anions are held together by ionic bonds. In solids, the ionic bonds are strong because the ions are close together. However, when ions are dispersed in water, the distance between them can be large; the strength of their attraction is thus greatly reduced. Under the conditions that exist in the cell, an ionic attraction is less than one-tenth as strong as a covalent bond that shares electrons equally (see Table 2.1).

Not surprisingly, ions with one or more units of charge can interact with polar molecules as well as with other ions. Such interaction results when table salt, or any other ionic solid, dissolves in water: "Shells" of water molecules surround the individual ions, separating them (Figure 2.11). The hydrogen bond that we described earlier is a type of ionic bond, because it is formed by electrical attractions. However, it is weaker than most ionic bonds because the hydrogen bond is formed by partial charges (5^+ and 8^-) rather than by whole-unit charges (+1 unit, -1 unit).

Polar and nonpolar substances interact best among themselves

"Like attracts like" is an old saying, and nowhere is it more true than in polar and nonpolar molecules, which tend to interact with their own kind. Just as water molecules interact with one another through their polarity-induced hydrogen bonds, any molecule that is itself polar will interact with other polar molecules by weak (5 + to 8 _) attractions in hydrogen bonds. If a polar molecule interacts with water in this way, it is called hydrophilic ("water-loving").

What about nonpolar molecules? For example, carbon (electronegativity 2.5) forms nonpolar bonds with hydrogen (electronegativity 2.1). The resulting hydrocarbon molecule—ethane—is nonpolar (Figure 2.12), and in water it will tend to aggregate with other nonpolar molecules rather than with polar water. Such molecules are called hydrophobic ("water-hating"), and the interactions between them are hydrophobic interactions. It is important to realize that hydrophobic substances do not really "hate" water; they can form weak interactions with it (recall that the electronegativities of carbon and hydrogen are not exactly the same).

SMALL MOLECULES: STRUCTURE AND BEHAVIOR 25

But these interactions are far weaker than the hydrogen bonds between the water molecules, and so the nonpolar substances keep to themselves.

These weak interactions between nonpolar substances are enhanced by van der Waals forces, which occur when two atoms are in close proximity. These forces result from random variations in the electron distribution in one mole-

Water

molecules

Ionic bonds between Na^+ and Cl^- hold ions together in a solid crystal.



a^*

9

v

gp «? *

Chloride ion -

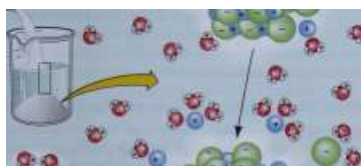
(CD

ffi Sodium ion - $\text{C}^{\oplus}(\text{Na}^+)$

£

Undissolved +

sodium - -v chloride +



$v \ll^o * -$

When NaCl is dissolved in water, the chloride anion (-) attracts the + pole of water..

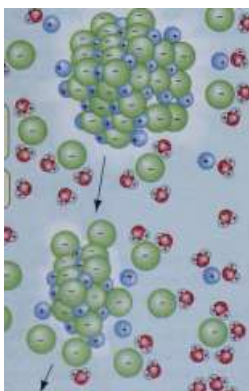
zs:

... and the sodium cation (+) attracts the - pole of water.

" ik a

ft

'<&\$*



Dissolved ions cannot reassociate into a solid.

Q o

y\

2.11 Water Molecules Surround Ions

When an ionic solid dissolves in water, polar water molecules cluster around cations or anions, blocking their reassociation into a solid and forming a solution.

The bonds between H and o in water are polar.



The bonds between H and C in ethane are not polar.



Water, a polar molecule Ethane, a nonpolar molecule (H z O) (CH 3 CH 3)

2.12 Polar and Nonpolar Molecules

Because the hydrocarbon ethane is nonpolar, it does not interact with water, but tends to interact with other nonpolar substances.

cule, which create an opposite charge distribution in the adjacent molecule. The result is a brief, weak attraction. Although each such interaction is brief and weak at any one site, the summation of many such interactions over the entire span of a large nonpolar molecule can produce substantial attraction, van der Waals forces are important in maintaining the structures of many biologically important substances.

Chemical Reactions: Atoms Change Partners

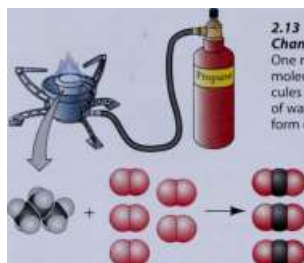
A chemical reaction occurs when atoms combine or change bonding partners. Consider the combustion reaction that takes place in the flame of a propane stove. When propane (C 3 H g) reacts with oxygen gas (o 2), the carbon atoms become bonded to oxygen atoms instead of to hydrogen atoms, and the hydrogen atoms become bonded to oxygen instead of carbon (Figure 2.13). As the covalently bonded atoms change partners, the composition of the matter changes, and propane and oxygen gas become carbon dioxide and water. This chemical reaction can be represented by the balanced equation

C 3 H £

+ 5O 2 ^3 Co 2 + 4 H 2 o

In this equation, the propane and oxygen are the reactants, and the carbon dioxide and water are the products. In this case, the reaction is complete: All the propane and oxygen are used up in forming the two products. The arrow symbolizes the chemical reaction. The numbers preceding the molecular formulas balance the equation and indicate how many molecules are used or are produced.

In this and all other chemical reactions, matter is neither created nor destroyed. The total number of carbons on the left equals the total number on the right. However, there is another product of this reaction: energy. The heat of the stove's flame and its blue light reveal that the reaction of propane and oxygen releases a great deal of energy. Energy is defined as the capacity to do work, but on a more intuitive level, it can be thought of as the capacity for change. Chemical reactions do not create or destroy energy, but changes in energy usually accompany chemical reactions.



2.7 3 Bonding Partners and Energy May Change in a Chemical Reaction

One molecule of propane reacts with five molecules of oxygen gas to give three molecules of carbon dioxide and four molecules of water. This reaction releases energy in the form of heat and light.

C_3H_8

$5 O_2$

Propane + Oxygen gas

Y Reactants

$3 CO_2$

Carbon dioxide

$4 H_2O$

+

+

+

9

<Sb

$4 H_2O$

Water

Y Products

In the reaction between propane and oxygen, the energy that was released as heat and light was already present in the reactants in another form, called potential energy. In some chemical reactions, energy must be supplied from the environment (for example, some substances will react only after being heated), and some of this supplied energy becomes stored as potential chemical energy in the bonds formed in the products.

We can measure the energy associated with chemical reactions using a unit called a calorie (cal). A calorie* is the amount of heat energy needed to raise the temperature of 1 gram of pure water from 14.5°C to 15.5°C. Another unit of energy that is increasingly used is the joule (J). When you compare data on energy, always compare joules to joules and calories to calories. The two units can be interconverted: 1 J = 0.239 cal, and 1 cal = 4.184 J. Thus, for example, 486 cal = 2,033 J, or 2.033 kJ. Although defined in terms of heat, the calorie and the joule are measures of any form of energy—mechanical, electric, or chemical.

Within living cells, chemical reactions called oxidation-reduction reactions take place. These biological reactions have much in common with the combustion of propane. The fuel is different (the sugar glucose, rather than propane), and the reactions proceed by many intermediate steps that permit the energy released from the glucose to be harvested and put to use by the cell. But the products are the same: carbon dioxide and water.

We will present and discuss energy changes, oxidation-reduction reactions, and several other types of chemical reactions that are prevalent in living systems in the chapters that follow.

The nutritionist's or dieter's Calorie, with a capital C, is what biologists call a kilocalorie (kcal) and is equal to 1,000 heat-energy calories.

+



Water: Structure and Properties

Water, like all other matter, can exist in three states: solid (ice), liquid, and gas (vapor) (Figure 2.14). Liquid water is the medium in which life originated on Earth more than 3.8 billion years ago, and it is in water that life evolved for its first billion years. Today, water covers three-fourths of Earth's surface, and the bodies of all active organisms contain between 45 and 95 percent water.

No organism can remain biologically active without water. Within cells, water participates directly in many chemical reactions, and it is the medium (or solvent) in which most biological reactions take place. In this section we will consider the structure

and interactions of water molecules, exploring how these generate properties essential to life.

Water has a unique structure and special properties

Each water molecule is composed of one oxygen atom bonded to two hydrogen atoms (H_2O). In the molecule, the four pairs of electrons in the outer shell of oxygen repel each other, producing a tetrahedral shape:

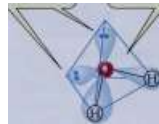
+ Heat and light

Energy J

+

These orbitals have non-bonding electron pairs.

The four orbitals are directed toward the corners of a tetrahedron.



The shape of the water molecule, its polar nature, and its capacity to form hydrogen bonds give water its unusual properties. For example, ice floats, and compared with other liquids, water is an excellent solvent, making it an ideal medium for biochemical reactions. Water is both cohesive (sticking to itself) and adhesive (sticking to other things). And the energy changes that accompany its transitions from solid to liquid to gas are significant in living systems.

ice floats. In its solid state (ice), water is held by its hydrogen bonds in a rigid, crystalline structure in which each water molecule is hydrogen-bonded to four others (Figure 2.15a). Although these molecules are held firmly in place, they are not as tightly packed as they are in liquid water (Figure 2.15b). In other words, solid water is less dense than liquid water, which is why ice floats in water.

If ice sank in water, as almost all other solids do in their corresponding liquids, ponds and lakes would freeze from



Solid water from a glacier floats in its liquid form. The clouds are also water, but not in its gaseous phase: They are composed of fine drops of liquid water.

the bottom up, becoming solid blocks of ice in winter and killing most of the organisms living in them. Once the whole pond had frozen, its temperature could drop well below the freezing point of water. However, because ice floats, it forms a protective insulating layer on the top of the pond, reducing heat flow to the cold air above. Thus fish, plants, and other organisms in the pond are not subjected to temperatures lower than 0°C , the freezing point of pure water.

melting and freezing. Compared with other nonmetallic substances of the same size, molecular ice requires a great

SMALL MOLECULES: STRUCTURE AND BEHAVIOR 27

deal of heat energy to melt. Melting 1 mole (a standard quantity— 6.02×10^{23} ; see page 28) of water molecules requires the addition of 5.9 kJ of energy. This value is high because more than a mole of hydrogen bonds must be broken for 1 mole of water to change from solid to liquid. In the opposite process, freezing, a great deal of energy must be lost for water to transform from liquid to solid. These properties help make water a moderator of temperature changes.

heat and cooling. Another property of water that moderates temperature is the high heat capacity of liquid water. The specific heat of a substance is the amount of heat energy required to raise the temperature of 1 gram of that substance by 1°C. Raising the temperature of liquid water takes a relatively large amount of heat because much of the heat energy is used to break the hydrogen bonds that hold the liquid together. Compared with other small molecules that are liquids, water has a high specific heat. This phenomenon contributes to the surprising constancy of the temperature of the oceans and other large bodies of water through the seasons of the year. The temperature changes of coastal land masses are also moderated by large bodies of water. Indeed, water helps minimize variations in atmospheric temperature throughout the planet.

evaporation and cooling. Water also has a high heat of vaporization, which means that a lot of heat is required to change water from its liquid state to its gaseous state (the process of evaporation). This heat is absorbed from the environment in contact with the water. Once again, much of the heat energy is used to break hydrogen bonds. Evaporation thus has a cooling effect on the environment— whether a leaf, a forest, or an entire land mass. This effect

- (a) Solid water (ice)
- (b) Liquid water
- (c) Gaseous water (steam)

A.. \$.ft f ,4

•<r -\$'■

""'..^."

a>

a

■ + ■ ■ ■

?

Q

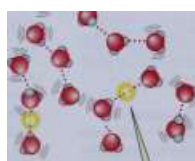
p

6

mfi q fi

'9>

o* i?"U*« '9>



In ice, water molecules are held in a rigid state by hydrogen bonds.

Hydrogen bonds continually break and form as water molecules move.



In its gaseous state, water does not form hydrogen bonds.

2.75 Hydrogen Bonds Hold Water Molecules Together

Hydrogen bonding exists between the molecules of water in both its liquid and solid states, (a) Solid water. (6) Liquid water. Although more structured, ice is less dense than liquid water, so it floats, (c) Water forms a gas when its hydrogen bonds are

broken and molecules move farther apart.

28 CHAPTER TWO

explains why sweating cools the human body: As sweat evaporates off the skin, it uses up some of the adjacent body heat.

cohesion and surface tension. In liquid water, the molecules are free to move about. The hydrogen bonds between the water molecules continually form and break. In other words, liquid water has a dynamic structure. On average, every water molecule forms 3.4 hydrogen bonds with other water molecules. This number represents fewer bonds than exist in ice, but it is still a high number.

These hydrogen bonds explain the cohesive strength of liquid water. The cohesive strength of water is what permits narrow columns of water to stretch from the roots to the leaves of trees more than 100 meters high. When water evaporates from leaves, the entire column moves upward in response to the pull of the molecules at the top.

Water also has a high surface tension, which means that the surface of liquid water exposed to the air is difficult to puncture. The water molecules in this surface layer are hydrogen-bonded to other water molecules below. The surface tension of water permits a container to be filled slightly above its rim without overflowing, and it permits small animals to walk on the surface of water (Figure 2.16).

Most biological substances are dissolved in water

A solution is produced when a substance is dissolved in water (an aqueous solution) or another liquid. Many of the important molecules in biological systems are polar, and therefore are soluble in water. Much of biochemistry takes place in an aqueous solution.

One branch of the study of solutions is qualitative analysis, which deals with substances dissolved in a solvent (in this case, water) and the chemical reactions that occur there. Qualitative analysis is the subject of much of the next few chapters.

Solutions can also be studied by quantitative analysis, in which concentrations—the amount of substance in a given amount of solution—are measured. What follows is a brief introduction to some of the quantitative chemical terms you will see in this text.

► A molecular formula uses chemical symbols to identify the different atoms in a compound, and subscript numbers to show how many of each type of atoms are present. Thus, the formula for sucrose—table sugar—is $C_{12}H_{22}O_{11}$.

► Each compound has a molecular weight (molecular mass) that is the sum of the atomic weights of all atoms in the molecule. Looking at the periodic table in Figure 2.1, you can calculate the molecular weight of table sugar to be approximately 342. Molecular weights are usually related to the molecule's size (Figure 2.17).

► A mole is the amount of an ion or compound in grams whose weight is numerically equal to its molecular weight. So one mole of sugar weighs 342 grams.

One aim of quantitative analysis is to study the behaviors of precise numbers of molecules in solution. But it is



2.76 Surface Tension

Water striders "skate" along, supported by the surface tension of the water that is their home.

not possible to count molecules directly. Instead, chemists use a constant that relates the weight of any substance to the number of molecules of that substance. This constant is called Avogadro's number, which is 6.02×10^{23} molecules per mole. It allows chemists to work with moles of substances (which can be weighed out in the laboratory) instead of actual molecules. The mole concept is analogous to the concept of a dozen: We buy a dozen eggs or a dozen doughnuts, knowing that we will get 12 of whichever we buy.

In the same way, chemists can dissolve a mole of sugar in water to make 1 liter, knowing that the mole contains 6.02×10^{23} individual sugar molecules. This solution—1 mole of a substance dissolved in water to make 1 liter—is called a 1 molar (1 M) solution.

The many molecules that dissolve in water in living tissues are not present at anything close to a 1 molar concentration. Most are in the micromolar (millionths of a mole; μM) to millimolar (thousandths of a mole; mM) range. Some, such as hormones,

are far less concentrated than this.

While these abbreviations seem to indicate very low concentrations, remember that even a 1 μM solution has 6.02×10^{17} molecules of the solute per liter.

Acids, Bases, and the pH Scale

Some substances dissolve in water and release hydrogen ions (H^+), which are actually single, positively charged protons. These tiny bits of charged matter can attach to other molecules, and in doing so, change their properties. In this section, we examine the properties of substances that release H^+ (called acids) and attach to H^+ (called bases). We will distinguish strong and weak acids and bases, and provide a quantitative means for stating the concentration of H^+ in solutions: the pH scale.

Acids donate H^+ ; bases accept it.

If hydrochloric acid (HCl) is added to water, it dissolves and ionizes, releasing the ions H^+ and Cl^- :



SMALL MOLECULES: STRUCTURE AND BEHAVIOR 29

2.17 Weights and Sizes of Atoms and Molecules

The color conventions used here are standard for the atoms. (Yellow is used for sulfur and phosphorus atoms, which are not depicted.)

Water is the solvent in which many biological reactions take place.

O

Hydrogen (H) Carbon (C) Nitrogen (N) Oxygen (O)

Molecular weights

1

12

14

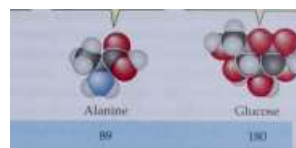
16

• 18

Water 18

Alanine is one of the building blocks of proteins.

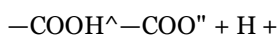
Glucose, a sugar, is an important food substance in most cells.



Because its H^+ concentration has increased, such a solution is acidic. Just like the burning reaction of propane and oxygen (see Figure 2.13), the dissolution of HCl to form its ions is a complete reaction. HCl is therefore called a strong

acid.

Acid releases H^+ ions in solution: HCl is an acid, as is H_2SO_4 (sulfuric acid). One molecule of sulfuric acid may ionize to yield two H^+ and one SO_4^{2-} . Biological compounds that contain $-\text{COOH}$ (the carboxyl group; see Figure 2.20) are also acids (such as acetic acid and pyruvic acid), because

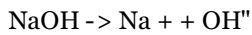


Not all acids dissolve fully in water. For example, if acetic acid is added to water, at the end of the reaction, there are not just the two ions, but some of the original acid as well. Because the reaction is not complete, acetic acid is a weak acid.

Like bases accept H^+ like acids, there are strong and weak

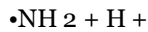
acids.

bases. If NaOH (sodium hydroxide) is added to water, the NaOH dissolves and ionizes, releasing OH^- and Na^+ ions:

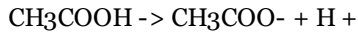


Because the concentration of OH^- increases, such a solution is basic, and because this reaction is complete, NaOH is a strong base.

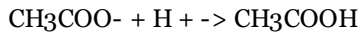
Weak bases include the bicarbonate ion (HCO_3^-), which can accept a H^+ and become carbonic acid (H_2CO_3), and ammonia (NH_3), which can accept a H^+ and become an ammonium ion (NH_4^+). Amino groups in biological molecules can also accept protons, acting as bases:



When acetic acid is dissolved in water, two reactions happen. First, acetic acid forms its ions:



Then, once ions are formed, they re-form acetic acid:



This pair of reactions is reversible. The formula for a reversible reaction can be written with two arrows:

A reversible reaction can proceed in either direction—left to right or right to left—depending on the relative starting concentrations of the reactants and products.

In principle, all chemical reactions are reversible. In terms of acids and bases, there are two types of reactions, depending on the extent of reversibility:

- Ionization of strong acids and bases is virtually irreversible.
- Ionization of weak acids and bases is somewhat reversible.

Many of the acid and base groups on large molecules in biological systems are weak.

Water is a weak acid

The water molecule has a slight but significant tendency to ionize into a hydroxide ion (OH^-) and a hydronium ion (H_3O^+). Actually, two water molecules participate in this ionization. One of the two molecules "captures" a hydrogen ion from the other, forming a hydroxide ion and a hydronium ion:



Water molecule (H_2O)

Water molecule (H_2O)

Hydroxide ion OH^- , a base

©



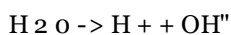
11

H

Hydronium ion H_3O^+ , an acid



The hydronium ion is in effect a hydrogen ion bound to a water molecule. For simplicity, biochemists tend to use a modified representation of the ionization of water:



The ionization of water is very important for all living creatures. This fact may seem surprising, since only about one water molecule in 500 million is ionized at any given time. But we are less surprised if we focus on the abundance of water in living systems and the reactive nature of the H^+ produced by ionization.

pH is the measure of hydrogen ion concentration

The terms "acid/c" and "basic" refer only to solutions. How acidic or basic a solution is depends on the relative concentrations of H^+ and OH^- ions in it. "Acid" and "base" refer

30 CHAPTER TWO

to compounds and ions. A compound or ion that is an acid can donate H^+ ; one that is a base can accept H^+ .

How do we specify how acidic or basic a solution is? First, let's look at the H^+ concentrations of a few contrasting solutions. In 1 liter of pure water, the H^+ concentration is 10^{-7} M. In 1 M hydrochloric acid, the H^+ concentration is 1 M; and in 1 M sodium hydroxide, the H^+ concentration is 10^{-14} M. Because its values range so widely, the H^+ concentration itself is an inconvenient quantity to measure. It is easier to work with the logarithm of the concentration, because logarithms compress this range.

We indicate how acidic or basic a solution is by its pH ("potential of Hydrogen"). The pH value is defined as the negative logarithm of the hydrogen ion concentration in moles per liter (molar concentration). In chemical notation, molar concentration is often indicated by putting square brackets around the symbol for a substance; thus $[H^+]$ stands for the molar concentration of H^+ . The equation for pH is

$$pH = -\log_{10} [H^+]$$

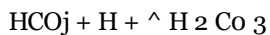
Since the H^+ concentration of pure water is 10^{-7} M, its pH is $-\log(10^{-7}) = -(-7)$, or 7. A smaller negative logarithm means a larger number. In practical terms, a lower pH means a higher H^+ concentration, or greater acidity. In 1 M HCl , the H^+ concentration is 1 M, so the pH is the negative logarithm of 1 ($-\log 10^0$), or 0. The pH of 1 M $NaOH$ is the negative logarithm of 10^{-14} , or 14.

A solution with a pH of less than 7 is acidic—it contains more H^+ ions than OH^- ions. A solution with a pH of 7 is neutral, and a solution with a pH value greater than 7 is basic. Figure 2.18 shows the pH values of some common substances.

Buffers minimize pH change

An organism must control the pH of the separate compartments within its cells. Animals must also control the pH of their blood. The normal pH of human blood is 7.4, and deviations of even a few tenths of a pH unit can be fatal. The control of pH is made possible in part by buffers—chemical systems that maintain a relatively constant pH even when substantial amounts of acid or base are added.

A buffer is a mixture of a weak acid and its corresponding base—carbonic acid (H_2CO_3) and bicarbonate ions (HCO_3^-). If acids are added to this buffer, not all the H^+ ions from that acid stay in solution. Instead, many of them combine with the bicarbonate ions to produce more carbonic acid. This reaction uses up some of the H^+ ions in the solution and decreases the acidifying effect of the added acid:



If a base is added, the reaction essentially reverses. Some of the carbonic acid ionizes to produce bicarbonate ions and more H^+ , which counteracts some of the added base. In this way, the buffer minimizes the effects of an added acid or base on pH. A given amount of acid or base causes a

Digital pH meter

Glass electrode



Sample being measured

H^+ concentration (moles per liter) 1

Stomach acid Lemon juice Vinegar, cola 3 Tomatoes 4

5

6 Human urine

Distilled water 7

Human blood

Seawater 8

Black coffee

Baking soda

Milk of magnesia

Household ammonia

9 10 11 12

Oven cleaner 13

14

Drain opener 15

10" 1

102

I 10~ 3 10" 4

A low pH is acidic.

Neutral pH

A change of 1 pH unit means a 10-fold change in H⁺ concentration.

A high pH is basic.



Basic

2.18 pH Values of Some Familiar Substances

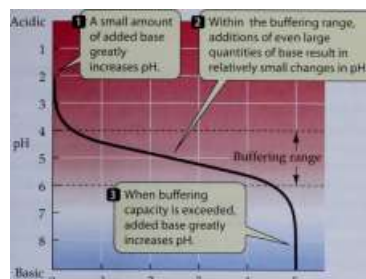
An electronic instrument similar to the one drawn at the top of the figure is used to measure the pH of a solution.

smaller change in pH in a buffered solution than in an unbuffered one (Figure 2.19).

Buffers illustrate an important chemical principle in reversible reactions called the law of mass action. Addition of a component on one side of a reversible system drives the reaction in the direction that uses up that compound. In this case, addition of an acid drives the reaction in one direction; addition of a base drives it in the other.

The Properties of Molecules

Some molecules are small, such as H₂ and CH₄. Others are larger, such as a molecule of table sugar (sucrose), which has 45 atoms. Still other molecules, such as proteins, are gigantic, sometimes containing tens of thousands of atoms bonded together in specific ways.



Basic

2 3 4

Amount of base added

2.7 9 Buffers Minimize Changes in pH

With increasing amounts of added base, the overall slope of a graph of pH is downward. In the buffering range, however, the slope is shallow. At high and low values of pH, where the buffer is ineffective, the slopes are much steeper.

Whether large, medium, or small, most of the molecules in living systems contain carbon atoms and are thus referred to as organic molecules. Most organic molecules include hydrogen and oxygen atoms as well as carbon, and many also include nitrogen and phosphorus.

All molecules have a specific three-dimensional shape. For example, the orientation of the bonding orbitals around the carbon atom gives the methane molecule (CH_4) the shape of a regular tetrahedron (see Figure 2.7c). In carbon dioxide (CO_2), the three atoms are in line. Larger molecules have complex shapes that result from the numbers and kinds of atoms present and the ways in which they are linked together. Some large molecules have compact, balllike shapes. Others are long, thin, ropelike structures. Their shapes relate to the roles these molecules play in living cells.

In addition to size and shape, molecules have certain properties that characterize them and determine their biological roles. Chemists use the characteristics of composition, structure (three-dimensional shape), reactivity, and solubility to distinguish a sample of one pure molecule from another. That certain groups of atoms are found together in a variety of different molecules simplifies our understanding of the reactions that molecules undergo in living cells.

Functional groups give specific properties to molecules

Functional groups are groups of atoms that make up part of a larger molecule and have particular chemical properties (shape, polarity, reactivity, solubility). The same functional group may be part of very different molecules. You will encounter several functional groups in your study of biology (Figure 2.20).

2.20 Some Functional Groups Important to Living Systems

These functional groups (highlighted in white boxes) are the most common ones found in biologically important molecules. R represents the "remainder" of the molecule, which may be any of a large number of carbon skeletons or other chemical group.



SMALL MOLECULES: STRUCTURE AND BEHAVIOR 31

An important kind of biological molecule containing functional groups is the amino acids, which have both a carboxyl group and an amino group attached to the same carbon atom, the α (alpha) carbon. Also attached to the α carbon atom are a hydrogen atom and a side chain, designated by the letter R:

r 'Side chain a Carbon-



COOH

Amino ^\ Carboxyl group g rou P

32 CHAPTER TWO

Different side chains have different chemical compositions, structures, and properties. Each of the 20 amino acids found in proteins has a different side chain that gives it its distinctive chemical properties, as we'll see in Chapter 3. Because they possess both carboxyl and amino groups, amino acids are simultaneously acids and bases. At the pH values commonly found in cells, both the carboxyl and the amino groups are ionized: The carboxyl group has lost a proton, and the amino group has gained one.

Isomers have different arrangements of the same atoms

Isomers are molecules that have the same chemical formula but different arrangements of the atoms. (The prefix "iso-" means "same" and is encountered in many biological terms.) Of the different kinds of isomers, we will consider two: structural isomers and optical isomers.

Structural isomers differ in how their atoms are joined together. Consider two simple molecules, each composed of 4 carbon and 10 hydrogen atoms bonded covalently, with the formula C_4H_{10} . These atoms can be linked together in two different ways, resulting in two forms of the molecule:

H H C H_3

$\text{H}_3\text{C}-\text{C}-\text{C}-\text{CH}_3$

II H H

Butane

$\text{H}_3\text{C}-\text{C}-\text{CH}_3$

I H

Isobutane

The different bonding relationships of butane and isobutane are distinguished in structural formulas, and the compounds have different chemical properties.

Many molecules of biological importance, particularly the sugars and amino acids, have optical isomers. Optical isomers occur whenever a carbon atom has four different atoms or groups attached to it. This pattern allows two different ways of making the attachments, each the mirror image of the other (Figure 2.21). Such a carbon atom is an asymmetric carbon, and the pair of compounds are optical isomers of each other. Your right and left hands are optical isomers. Just as a glove is specific for a particular hand, some biochemical molecules can interact with one optical isomer of a compound, but are unable to "fit" the other.

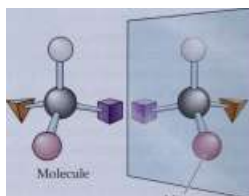
The a carbon in an amino acid is an asymmetric carbon because it is bonded to four different functional groups. Therefore, amino acids exist in two isomeric forms, called D-amino acids and L-amino acids, "d" and "l" are abbreviations for the Latin terms for right (dextro) and left (levo), respectively. Only L-amino acids are commonly found in most organisms.

Between the small molecules we have discussed in this chapter and the world of the living cell stands another level, that of the macromolecules. These huge molecules— the proteins, lipids, carbohydrates, and nucleic acids—are the subject of the next chapter.



Hand

Mirror image



Molecule

Mirror image

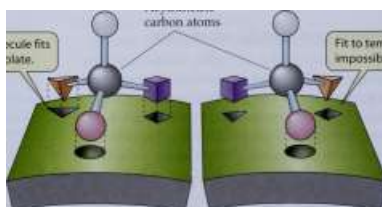
(b)

2.21 Optical Isomers

(a) Optical isomers are mirror images of each other, (b) Molecular optical isomers result when four different groups are attached to a single carbon atom, (c) If a template is laid out to match the groups on one carbon atom, the groups on the mirror-image isomer cannot be rotated to fit the same template.

Molecule fits template.

Asymmetric carbon atoms



Fit to template is impossible for isomer.

Chapter Summary

Atoms: The Constituents of Matter

► Matter is composed of atoms. Each atom consists of a positively charged nucleus of protons and neutrons, surrounded by electrons bearing negative charges.

- ▶ There are many elements in nature, but only a few of them make up the bulk of living systems. Review Figure 2.1
- ▶ Isotopes of an element differ in their numbers of neutrons. Some isotopes are radioactive, emitting radiation as they decay. Review Figure 2.2
- ▶ Electrons are distributed in shells consisting of orbitals. Each orbital contains a maximum of two electrons. Review Figures 2.4, 2.5
- ▶ In losing, gaining, or sharing electrons to become more stable, an atom can combine with other atoms to form molecules. Review Table 2.1

Chemical Bonds: Linking Atoms Together

- ▶ Covalent bonds are strong bonds formed when two atomic nuclei share one or more pairs of electrons. Covalent bonds have spatial orientations that give molecules three-dimensional shapes. Review Figures 2.6, 2.7, Table 2.2
- ▶ Nonpolar covalent bonds are formed when the electronegativities of two atoms are approximately equal. When atoms with strong electronegativity (such as oxygen) bond to atoms with weaker electronegativity (such as hydrogen), a polar covalent bond is formed, in which one end is δ^- and the other is δ^+ . Review Figure 2.8, Table 2.3
- ▶ Hydrogen bonds are weak electrical attractions that form between a δ^+ hydrogen atom in one molecule and a δ^- nitrogen or oxygen atom in another molecule or in another part of a large molecule. Hydrogen bonds are abundant in water. Review Figure 2.9
- ▶ Ions are electrically charged bodies that form when an atom gains or loses one or more electrons. Ionic bonds are electrical attractions between oppositely charged ions. Ionic bonds are strong in solids, but weaker when the ions are separated from one another in solution. Review Figures 2.10, 2.11
- ▶ Nonpolar molecules do not interact directly with polar substances, including water. Nonpolar molecules are attracted to each other by very weak bonds called van der Waals forces. Review Figure 2.12

Chemical Reactions: Atoms Change Partners

- ▶ In chemical reactions, substances change their atomic compositions and properties. Energy is released in some reactions, whereas in others energy must be provided. Neither matter nor energy is created or destroyed in a chemical reaction, but both change form.
- ▶ Combustion reactions are oxidation-reduction reactions in which a fuel is converted to carbon dioxide and water, while energy is released as heat and light. In living cells, combustion reactions take place in multiple steps so that the released energy can be harvested for cellular activities. Review Figure 2.13

Water: Structure and Properties

- ▶ Water's molecular structure and its capacity to form hydrogen bonds give it unusual properties that are significant for life. Water is an excellent solvent; solid water floats in liquid water; and water gains or loses a great deal of heat when it changes its state, a property that moderates environmental temperature changes. Review Figure 2.15
- ▶ The cohesion of water molecules permits liquid water to rise to great heights in narrow columns and produces a high surface tension. Water's high heat of vaporization assures effective cooling when water evaporates.
- ▶ Solutions are produced when substances dissolve in water. The concentration of a solution is the amount of a given substance in a given amount of solution. Most biological substances are dissolved in water at very low concentrations.

Acids, Bases, and the pH Scale

- ▶ Acids are substances that donate hydrogen ions (H^+). Bases are substances that accept hydrogen ions.
- ▶ The pH of a solution is the negative logarithm of the hydrogen ion concentration. Values lower than pH 7 indicate an acidic solution; values above pH 7 indicate a basic solution. Review Figure 2.18
- ▶ Buffers are systems of weak acids and bases that limit the change in pH when hydrogen ions are added or removed. Review Figure 2.19

The Properties of Molecules

- ▶ Molecules vary in size, shape, reactivity, solubility, and other chemical properties.
- ▶ Functional groups make up part of a larger molecule and have particular chemical properties. The consistent chemical behavior of functional groups helps us understand the properties of the molecules that contain them. Review Figure 2.20
- ▶ Structural and optical isomers have the same kinds and numbers of atoms, but differ in their structures and properties. Review Figure 2.21

For Discussion

1. Would you expect the elemental composition of Earth's crust to be the same as that of the human body? How could you find out?
2. Lithium (Li) is the element with atomic number 3. Draw the electronic structures of the Li atom and of the Li⁺ ion.
3. Draw the structure of a pair of water molecules held together by a hydrogen bond. Your drawing should indicate the covalent bonds.
4. The molecular weight of sodium chloride (NaCl) is 58.45. How many grams of NaCl are there in 1 liter of a 0.1 M NaCl solution? How many in 0.5 liter of a 0.25 M NaCl solution?
5. The side chain of the amino acid glycine is simply a hydrogen atom (—H). Are there two optical isomers of glycine? Explain.

Self-quizzes and Supplemental Readings for each chapter are on the Student Web Site/CD-ROM.



Macromolecules:

Their Chemistry and Biology

Make A SPIDER WEB IS AN AMAZING STRUCTURE. NOT JUST BECAUSE IT IS BEAUTIFUL TO LOOK AT, BUT IT IS AN ARCHITECTURAL WONDER THAT SERVES AS THE SPIDER'S HOME, ITS MATING GROUND, AND ITS MEANS OF HUNTING AND CAPTURING FOOD. Consider a fly that happens to intersect with a spider web. The fibers of the web must slow down the fly, but they cannot break, so they must stretch in order to dissipate the energy of the fly's movement. On the other hand, the fibers holding the web together cannot stretch too much, because they must be strong enough to hold the entire structure in place and not let the web wobble out of control. Web fibers are far thinner than human hair, yet they are stronger than steel and, in some cases, more elastic than nylon. In fact, spider silk may be as strong as Kevlar, a synthetic substance used to make bulletproof vests and the cords attached to parachutes.

Spider silk is composed of slight variations on a single type of huge molecule—a macromolecule—called a protein. The many types of proteins in biological systems are composed of different amounts of the 20 molecules known as amino acids, and spider silks have their own unique selections of these molecules. The silk protein that stretches contains amino acids that allow it to curl into a spiral, and when these spirals associate into silk fibers, they can slip along each other to change the fiber's length. The strong fibers, in contrast, are made up of amino acids that fold the individual proteins into flat sheets, with ratchets that fit parallel sheets together (much like Lego blocks), so that the fibers are hard to pull apart. The relationship between chemical structure and biological function is the theme of this chapter and many of the succeeding ones in this section. The four major types of biological macromolecules—proteins, carbohydrates, lipids, and nucleic acids—are composed of building blocks called monomers. In the case of proteins like spider silk, the monomers are amino acids; carbohydrate monomers are sugars, and nucleic acid monomers are nucleotides. Some lipids are composed of a small molecule, glycerol, covalently bonded to larger fatty acids. Lipids interact to form huge macromolecular aggregates, such as the membranes that surround cells.

The four kinds of large molecules are made the same way in all living things, and are present in roughly the same proportions in all organisms (Figure 3.1). Although an

A Complex Macromolecule

Spider silk (purple) being spun into web material from a gland by the shiny black spider, *Castercantha*.

An apple tree is obviously different from a person, their basic chemistry is the same, demonstrating the unity of life. A protein that has a certain role in the apple probably has a similar role in the human. One important advantage of biochemical unity is that organisms can eat one another. When you eat an apple, the molecules you take in include carbohydrates, lipids, and proteins that can be re-fashioned into the special varieties of those molecules used by humans.



Macromolecules: Giant Polymers

Macromolecules are giant polymers (poly-, "many"; -mer, "unit") constructed by the covalent linking of smaller molecules called monomers (Table 3.1). These monomers may or may not be identical, but they always have similar chemical structures. Molecules with molecular weights exceeding 1,000 are usually considered macromolecules, and the proteins, polysaccharides (large carbohydrates), and nucleic acids of living systems certainly fall into this category.

Each type of macromolecule performs some combination of a diversity of functions: energy storage, structural

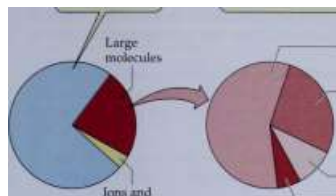


MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 35

Living tissues are 70% water.

Four kinds of large molecules are present in roughly the same proportions in all living things.

— t'



Ions and

small molecules

r Proteins

(polypeptides)

-Nucleic acids

Carbohydrates (polysaccharides)

Lipids

3. 1 Substances Found in Living Tissues

The substances shown here make up the nonmineral components of living tissue (bone would be an example of a "mineral tissue"). Most tissues are at least 70 percent water.

support, protection, catalysis, transport, defense, regulation, movement, and heredity. These roles are not necessarily exclusive. For example, both carbohydrates and proteins can play structural roles, supporting and protecting tissues and organisms. However, only nucleic acids specialize in information storage and function as hereditary material, carrying both species and individual traits from generation to generation.

The functions of macromolecules are directly related to their shapes and to the chemical properties of their monomers. Some macromolecules, such as catalytic and defensive proteins, fold into compact spherical forms with surface features that make them water-soluble and capable of intimate interaction with other molecules. Other proteins and carbohydrates form long, fibrous systems that provide strength and rigidity to cells and organisms. Still other long, thin assemblies of proteins can contract and cause movement.

Because macromolecules are so large, they contain many different functional groups (see Figure 2.20). For example, a large protein may contain hydrophobic, polar, and charged functional groups that give specific properties to local sites on a macromolecule. As we will see, this diversity of properties determines the shapes of macromolecules and their interactions with both other macromolecules and smaller molecules.

^ ^ The Building Blocks of Organisms

MONOMER

SIMPLE POLYMER

COMPLEX POLYMER (MACROMOLECULE)

Amino acid

Nucleotide Monosaccharide (sugar)

Peptide or

oligopeptide Oligonucleotide Oligosaccharide

Polypeptide (protein)

Nucleic acid Polysaccharide (carbohydrate)

Condensation Reactions

The polymers of living things are constructed from monomers by a series of reactions called condensation reactions or dehydration reactions (both words refer to the loss of water). Condensation reactions result in covalently bonded monomers (Figure 3.2a). The condensation reactions that produce the different kinds of macromolecules differ in detail, but in all cases, polymers will form only if energy is added to the system. In living systems, specific energy-rich molecules supply this energy.

The reverse of a condensation reaction is a hydrolysis reaction (hydro-, "water"; -lysis, "break"). These reactions digest polymers and produce monomers. Water reacts with the bonds that link the polymer together, and the products are free monomers. The elements (H and O) of H_2O become part of the products (Figure 3.2b). Like condensation reactions, hydrolysis requires the addition of energy.

We begin our study of biological macromolecules with a very diverse group of polymers, the proteins.

(a) Condensation

Monomer

$OH-H-Monomer$

$OH-H-Monomer-OH$

H_2O



Water is removed in condensation.

$H-Monomer$

$Monomer-OH$

H

Monomer

OH

A covalent bond forms between monomers.

H_2O

H

Monomer

$Monomer-Monomer$

$-OH$

Condensation links monomers together, forming polymers.

(b) Hydrolysis

OH

H

Monomer

$OH-H-Monomer$

Monomer —OH

A covalent bond between monomers is broken.

H₂O-

H — Monomer - OH H — Monomer

OH H

Monomer - OH

Hydrolysis breaks polymers apart, resulting in free monomers.

3.2 Condensation and Hydrolysis of Polymers

(a) A condensation reaction links monomers into polymers.

(b) A hydrolysis reaction digests polymers into individual monomers. In living tissues, these reactions do not occur spontaneously, but require added energy.

36 CHAPTER THREE

Proteins: Polymers of Amino Acids

Proteins are involved in structural support, protection, catalysis, transport, defense, regulation, and movement. Among the functions of macromolecules listed earlier, only energy storage and heredity are not usually performed by proteins.

Of particular importance are proteins called enzymes that increase the rates of chemical reactions in cells, a function known as catalysis. In general, each chemical reaction requires a different enzyme, because proteins show great specificity for the smaller molecules with which they interact.

Proteins range in size from small ones such as the RNA-digesting enzyme ribonuclease A, which has a molecular weight of 5,733 and 51 amino acid residues, to huge molecules such as the cholesterol transport protein apolipoprotein B, which has a molecular weight of 513,000 and 4,636 amino acid residues. (The word "residue" refers to a monomer when it is part of a polymer.) Each of these proteins consists of a single chain of amino acids (a polypeptide chain) folded into a specific three-dimensional shape that is required for protein function.

Some proteins have more than one polypeptide chain. For example, the oxygen-carrying protein hemoglobin has four chains that are folded separately and associate together to make the functional protein. As we will see later in this book, there are many such "multi-protein machines," composed of dozens of interacting polypeptides.

Each of these proteins has a characteristic amino acid composition. But not every protein contains all kinds of amino acids, nor an equal number of different ones. The diversity in amino acid content and sequence is the source of the diversity in protein structures and functions. In some cases, additional chemical structures called prosthetic groups may be attached covalently to the protein. These groups include carbohydrates, lipids, phosphate groups, the iron-containing heme group that binds to hemoglobin, and metal ions such as copper and zinc. Prosthetic groups are discussed further in Chapter 6.

The next several chapters will describe the many functions of proteins. To understand them, we must first explore protein structure. First, we will examine the properties of the amino acids and how they link to form proteins. Then we will systematically examine protein structure and look at how a linear chain of amino acids is consistently folded into a compact three-dimensional shape. Finally, we will see how this structure provides a specific physical and chemical environment for other molecules that can interact with the protein.

Proteins are composed of amino acids

The 20 amino acids commonly found in proteins have a wide variety of properties. In Chapter 2, we looked at the structure of amino acids and identified four different groups attached to a central (α) carbon atom: a hydrogen atom, an amino group (NH₃), a carboxyl group (COO⁻), and a side chain, or R group. The R groups (R stands for

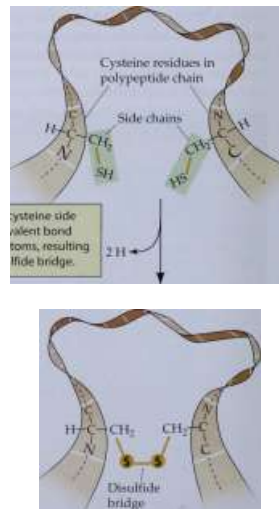
"remainder" or "residue") of amino acids are important in determining the three-dimensional structure and function of the macromolecule. They are highlighted in white in Table 3.2.

As Table 3.2 shows, amino acids are grouped and distinguished by their side chains. Some side chains are electrically charged (+1, -1), while others are polar (δ+, δ-) or nonpolar and hydrophobic.

- ▶ The five amino acids that have electrically charged side chains attract water and oppositely charged ions of all sorts.
- ▶ The five amino acids that have polar side chains tend to form weak hydrogen bonds with water and with other polar or charged substances.
- ▶ Seven amino acids have side chains that are nonpolar hydrocarbons or very slightly modified hydrocarbons. In the watery environment of the cell, the hydrophobic side chains may cluster together.
- ▶ Three amino acids—cysteine, glycine, and proline—are special cases, although their R groups are generally hydrophobic.

The cysteine side chain, which has a terminal —SH group, can react with another cysteine side chain to form a covalent bond called a disulfide bridge (—S—S—) (Figure 3.3). Disulfide bridges help determine how a protein chain folds. When cysteine is not part of a disulfide bridge, its side chain is hydrophobic.

The —SH groups of two cysteine side chains react to form a covalent bond between the two sulfur atoms, resulting in the formation of a disulfide bridge.



Disulfide bridge

3.3 A Disulfide Bridge

Disulfide bridges (—S—S—) are important in maintaining the proper three-dimensional shapes of some protein molecules.

MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 37

Twenty Amino Acids Found in Proteins

Amino acids have both three-letter and single-letter abbreviations.

A. Amino acids with electrically charged side chains

Positive ©

Arginine (Arg) (R)

H

H₂N

CH₂, I CH₂

CH₂

NH₂ + C=NH₂

NH₂,

Histidine (His) (H)

H

-W-COOH₃N⁺-»-COO-

CH₂

I ~ -C—NH

CH

/ HC — M₁

Lysine (Lys) (K)

H

H₃N⁺ - < ^-

COO⁻

CH₂

I CH₂

I CH₂

I CH₂

I + NH₃⁺

Negative charge

Aspartic acid (Asp) (D)

The general

structure of all

amino acids is

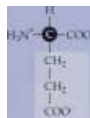
the same...

H₃N⁺ - W - COO⁻

... but each has a different side chain.



Glutamic acid (Glu) (E)



B. Amino acids with polar but uncharged side chains C. Special cases

Serine Threonine Asparagine Glutamine Tyrosine Cysteine

(Ser)(S) (Thr)(T) (Asn) (N) (Gln) (Q) (Tyr) (Y) (Cys) (C)

H H

H₃N⁺ - W - COO⁻ H₃N⁺ - W - COO⁻ - CH₂OH H₃N⁺ - W - COO⁻ - CH₂OH

Glycine Proline

(Gly) (G) (Pro) (P)

H

H H

H₃N⁺ - COO⁻ H₃N⁺ - W - COO⁻ H₃N⁺ - W - COO⁻ H₃N⁺ - W - COO⁻

rn_ fH. rw. ' ^

CH₂

CH₂

I "

H₃N⁺ O



CH₂

OH

CH₂ I SH

When amino acids polymerize, the carboxyl group of one amino acid reacts with the amino group of another, undergoing a condensation reaction that forms a peptide linkage. Figure 3.4 gives a simplified description of this reaction. (In reality, other molecules must activate the amino acids in order for this reaction to proceed, and there are intermediate steps in the process. We will examine these in Chapter 12).

H

H-

+

In-

H

H

I

I

D

o

II c-

o

Amino group

H

I H — N-I H

H

I

•

"V

o

H₂O

O"

Carboxyl group

H

H-

— N

H

N terminus

Peptide linkage

H O

I II , @ —C —N-

h H

The amino and carboxyl groups of two amino acids react to form a peptide linkage. A molecule of water is lost (condensation) as each linkage forms.

H I

□

v

C terminus

Repetition of this reaction links many amino acids together into a polypeptide.

3.4 Formation of Peptide Linkages

In living things, the reaction leading to a peptide linkage has many intermediate steps, but the reactants and products are the same as those shown in this simplified diagram.

Just as a sentence begins with a capital letter and ends with a period, polypeptide chains have a linear order. The chemical "capital letter" marking the beginning of a polypeptide chain is the amino group of the first amino acid in the chain and is known as the N terminus. The chemical punctuation mark for the end of the chain is the carboxyl group of the last amino acid (the C terminus).

All the other amino and carboxyl groups (except those in side chains) are involved in peptide bond formation, so they do not exist in the chain as "free," intact groups. Biochemists refer to the "N → C," or "amino-to-carboxyl" orientation of polypeptides.

The peptide linkage has two characteristics that are important in the three-dimensional structure of proteins. First, in many single covalent bonds, the groups on either side of the bonds are free to rotate in space. This is not so with the C—N peptide bond. The adjacent atoms (the α carbons of the two adjacent amino acids) are not free to rotate because of the partial double-bond character of the peptide bond. Chemists will realize that this is due to the resonance between the strong electronegativity of the oxygen bound to the carbon and the weak electronegativity of the hydrogen bound to the nitrogen. This characteristic limits the folding of the polypeptide.

Second, the oxygen bound to the carbon carries a slight negative charge (δ⁻), whereas the hydrogen bound to the nitrogen is slightly positive (δ⁺). This asymmetry of charge favors hydrogen bonding within the protein molecule itself and with other molecules, contributing to both the structure and the function of many proteins.

The primary structure of a protein is its amino acid sequence

There are four levels of protein structure, called primary, secondary, tertiary, and quaternary. The precise sequence of amino acids in a polypeptide constitutes the primary structure of a protein (Figure 3.5a). The peptide backbone of this primary structure consists of a repeating sequence of three atoms (—N—C—C—): the N from the amino group, the α carbon, and the C from the carboxyl group of each amino acid. Scientists have deduced the primary structure of many proteins, and use the single-letter abbreviations for amino acids (see Table 3.2) to record the sequence. Here, for example, are the first 25 amino acids (out of a total of 457) for the protein hexokinase, from baker's yeast:

AASXDXSLVEVHXXVFIVPPXILQA

The theoretical number of different proteins is enormous. Since there are 20 different amino acids, there are $20 \times 20 = 400$ distinct dipeptides (two linked amino acids), and $20 \times 20 \times 20 = 8,000$ different tripeptides (three linked amino acids). Imagine this process of multiplying by 20 extended to a protein made up of 100 amino acids (which is considered a small protein). There could be 20^{100} such small proteins, each with its own distinctive primary structure. How large is the number 20^{100} ? There aren't that many electrons in the entire universe!

At the higher levels of protein structure, local coiling and folding give the molecule its final functional shape, but all of these levels derive from the primary structure—that is, which amino acids are at which locations on the polypeptide chain. The properties associated with a precise sequence of amino acids determine how the protein can twist and fold, thus adopting a specific stable structure that distinguishes it from every other protein.

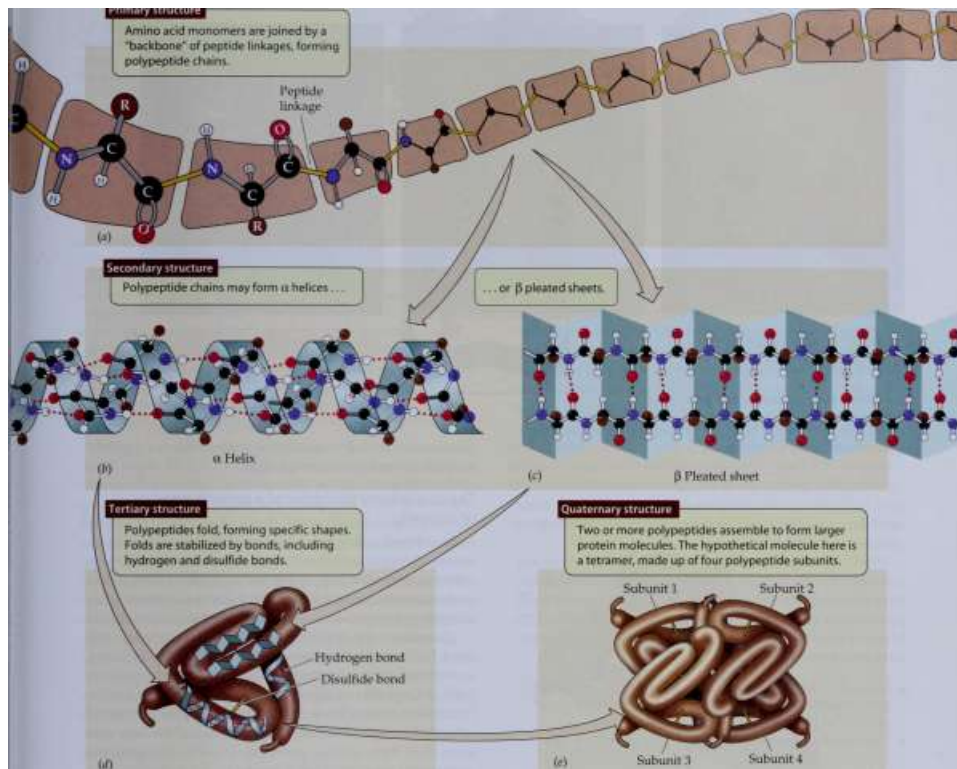
The secondary structure of a protein requires hydrogen bonding

Although the primary structure of each protein is unique, the secondary structures of many different proteins may be quite similar. A protein's secondary structure consists of regular, repeated patterns in different regions of a polypeptide chain. There are two basic types of secondary structure, both of them determined by hydrogen bonding between the amino acid residues that make up the primary structure.

the α helix. The α (alpha) helix is a right-handed coil that is "threaded" in the same direction as a standard wood screw (Figure 3.5b). The R groups extend outward from the peptide backbone of the helix. The coiling results from hydrogen bonds between the slightly positive hydrogen of the N—H of one amino acid residue and the slightly negative oxygen of the C=O of another. When this pattern of hydrogen bonding is established repeatedly over a segment of the protein, it stabilizes the coil, resulting in an α helix. Amino acids with large R groups that distort the coil or

MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 39

Primary structure



/ Subunit 3

Subunit 4

3.5 The Four Levels of Protein Structure

Secondary, tertiary, and quaternary structure all arise from the primary structure of the protein.

otherwise prevent the formation of the necessary hydrogen bonds will keep the α helix from forming.

Alpha-helical secondary structure is particularly evident in the insoluble fibrous structural proteins called keratins, which make up hair, hooves, and feathers. Hair can be stretched because stretching requires that only the hydrogen bonds of the α helix, not the covalent bonds, be broken; when the tension on the hair is released, both the hydrogen bonds and the helix reform.

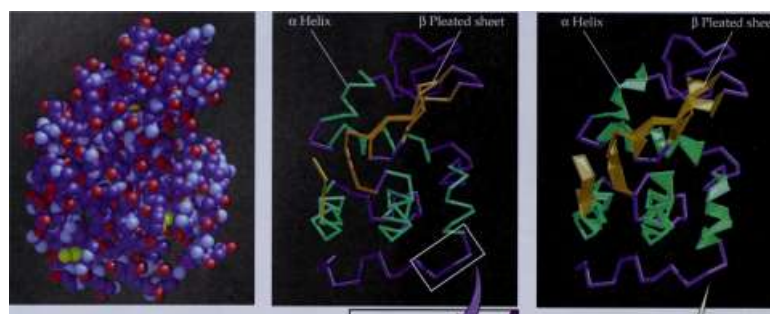
the β pleated sheet. The β (beta) pleated sheet is formed from two or more polypeptide chains that are almost com-

pletely extended and lying next to one another. The sheet is stabilized by hydrogen bonds between the N—H groups on one chain and the C=O groups on the other (Figure 3.5c). A β pleated sheet may form between separate polypeptide chains, as in spider silk, or between different regions of the same polypeptide that is bent back on itself. Many proteins contain regions of both a helix and β pleated sheet in the same polypeptide chain.

The tertiary structure of a protein is formed by bending and folding

In many proteins, the polypeptide chain is bent at specific sites and folded back and forth, resulting in the tertiary structure of the protein (Figure 3.5d). Although the α helices and β pleated sheets contribute to the tertiary struc-

40 CHAPTER THREE



A realistic depiction of lysozyme shows dense packing of its atoms.

3.6 Three Representations of Lysozyme

Different molecular representations of a protein emphasize different aspects of its tertiary structure. These three representations of lysozyme are similarly oriented.

ture, only parts of the macromolecule usually have these secondary structures, and large regions consist of structures unique to a particular protein.

While hydrogen bonding is responsible for secondary structure, the interactions between R groups determine tertiary structure. We described the various strong and weak interactions between atoms in Chapter 2 (see Table 2.1). Many of these interactions are involved in determining tertiary structure:

- Covalent disulfide bridges can form between specific cysteine residues (see Figure 3.3), holding a folded polypeptide in place.
- Hydrophobic side chains can aggregate together in the interior of the protein, away from water, folding the polypeptide in the process.
- Van der Waals forces can stabilize the close interactions between the hydrophobic residues.
- Ionic interactions can occur between positively and negatively charged side chains buried deep within a protein, away from water, forming a salt bridge.

A complete description of a protein's tertiary structure specifies the location of every atom in the molecule in three-dimensional space, in relation to all the other atoms. The tertiary structure of the protein lysozyme is represented in Figure 3.6.

Bear in mind that both tertiary structure and secondary structure derive from the protein's primary structure. If lysozyme is heated slowly, the heat energy will disrupt only the weak interactions and cause only the tertiary structure to break down. But the protein will return to its normal tertiary structure when it cools, demonstrating that all the

^^

The "backbone" of lysozyme consists of repeating N—C—C units of amino acids.

information needed to specify the unique shape of a protein is contained in its primary structure.

The quaternary structure of a protein consists of subunits

As mentioned earlier, many functional proteins have two or more polypeptide chains, called subunits, each of them folded into its own unique tertiary structure. The protein's quaternary structure results from the ways in which these multiple polypeptide subunits bind together and interact.

Quaternary structure is illustrated by hemoglobin (Figure 3.7). Hydrophobic interactions, van der Waals forces, hydrogen bonds, and ionic bonds all help hold the four subunits together to form the hemoglobin molecule. The function of hemoglobin is to carry oxygen in red blood cells. As hemoglobin binds one O₂ molecule, the four sub-units shift their relative positions slightly, changing the quaternary structure. Ionic bonds are broken, exposing buried side chains that enhance the binding of additional O₂ molecules. The structure changes again when hemoglobin releases its oxygen molecules to the cells of the body.

The surfaces of proteins have specific shapes

Small molecules in a solution are in constant motion. They vibrate, rotate, and move from place to place like corn in a popper. If two of them collide in the right circumstances, a chemical reaction can occur. The specific shapes of proteins allow them to bind noncovalently with other molecules, which in turn allows other important biological events to occur. For example:

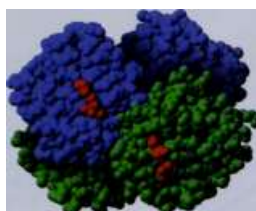
- Two adjacent cells can stick together because proteins protruding from each of the cells interact with each other (see Chapter 5).
- A substance can enter a cell by binding to a carrier protein in the cell surface membrane (see Chapter 5).

(«)

a Subunits

3.7 Quaternary Structure of a Protein

Hemoglobin consists of four folded polypeptide subunits that assemble themselves into the quaternary structure shown here. In these two graphic representations, each type of sub-unit is a different color. The heme groups contain iron and are the oxygen-carrying sites.





P Subunits

Heme

- ▶ A chemical reaction can be speeded up when an enzyme protein binds to one of the reactants (see Chapter 6).
- ▶ A multi-protein "machine," DNA polymerase, can catalyze the replication of DNA (see Chapter 11).
- ▶ Another multi-protein "machine," the ribosome, can synthesize proteins (see Chapter 12).
- ▶ Proteins on a cell's outer surface can bind to chemical signals such as hormones (see Chapter 15).
- ▶ Defensive proteins called antibodies can recognize the shape of a virus coat and bind to it (see Chapter 19).

When a small molecule collides with and binds to a much larger protein, it is like a baseball being caught by a catcher: The catcher's mitt has a shape that binds to the ball and fits around it. A hockey puck or a ping-pong ball would not fit the baseball mitt. Thus, the binding of a small molecule to a protein involves a general interaction between two three-dimensional objects that becomes more specific after initial binding. When two large polypeptide chains bind to each other, the interactions are more complicated because extensive surfaces of each macromolecule must come into contact, but the principle is the same.

Biological specificity depends not just on the shape of a protein, but also on the surface chemical groups that it presents to a substance attempting to bind to it (Figure 3.8). The groups on the surface are the R groups of the exposed amino acids, and are therefore a property of the protein's primary structure.

Look again at the structures of the 20 amino acids in Table 3.2, noting the properties of the R groups. Exposed hydrophobic groups will bind to similarly nonpolar groups in the substance with which the protein interacts (often called the ligand). Charged R groups will bind to oppositely charged groups on the ligand. Polar R groups containing a hydroxyl ($-\text{OH}$) group can form a hydrogen bond with an incoming ligand. These three types of interactions—hydrophobic, ionic, and hydrogen bonding—are weak by themselves, but strong when all of them act together. So the exposure of appropriate amino acid R groups on the protein surface allows specific binding of a ligand to occur.

Protein shapes are sensitive to the environment

The three-dimensional structure of a protein determines what it binds and, therefore, its function. The primary structure of a protein constrains its secondary, tertiary, and (if subunits exist) quaternary structures. A major effort in

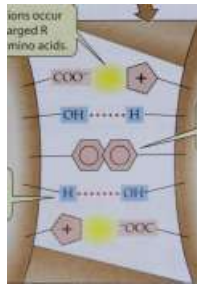
biochemistry is to try to predict three-dimensional protein structure from amino acid sequence. For some short sequences, this is relatively straightforward: For example, certain amino acid sequences will fold into a β pleated sheet. But for large polypeptide chains, the multitude of potential interactions make structure prediction a problem, approachable only by computer. Indeed, a whole new field of computational biochemistry has emerged to tackle the challenge of structure prediction.

Knowing the exact shape of a protein and what can bind to it is important not only in understanding basic biology, but in applied fields such as medicine as well. For example, the three-dimensional structure of a protease, a protein es-



Ionic attractions occur between charged R groups on amino acids

Hydrogen bonds form between two polar groups.



Two nonpolar groups interact hydrophobically.

3.8 Noncovalent Interactions between Polypeptides and Other Molecules

Noncovalent interactions allow a protein to bind tightly to another molecule with specific properties, or allow regions within a protein to interact with one another.

42 CHAPTER THREE

Denaturing agents can disrupt the tertiary and secondary structure of a protein and destroy the protein's biological functions.



Denatured protein

Native protein



Renaturing (reassembly into a functional protein) is sometimes possible, but usually denaturation is irreversible.

V

3.9 Denaturation Is the Loss of Tertiary Protein Structure and Function

Agents that can cause denaturation include high temperatures and certain chemicals.

essential for the replication of HIV—the virus that causes AIDS—was first determined in this way. Then specific inhibitors were designed to interact with its surface. These protease inhibitors have prolonged the lives of countless people living with HIV.

Because it is determined by weak forces, protein shape is sensitive to environmental conditions that would not break covalent bonds but do upset weaker noncovalent interactions. Elevated temperatures, pH changes, or altered salt concentrations can cause a protein to adopt a different, biologically inactive tertiary structure. Increases in temperature cause more rapid molecular movements and thus can break hydrogen bonds and hydrophobic interactions. Alterations in pH can change the pattern of ionization of carboxyl and amino groups in the R groups of amino acids, thus disrupting the pattern of ionic attractions and repulsions that contributes to normal tertiary structure.

The loss of normal tertiary structure is called denaturation, and it is always accompanied by a loss of the normal biological function of the protein (Figure 3.9). Denaturation

can be caused by heat or by high concentrations of polar substances such as urea, which disrupt the hydrogen bonding that is crucial to protein structure. Nonpolar solvents may also disrupt normal structure.

Usually denaturation is irreversible, because amino acids that were buried may now be exposed and vice versa, causing a new structure to form or different molecules to bind to the protein. Boiling an egg denatures its proteins and is, as you know, not reversible. However, as we saw earlier, denaturation is often reversible in the laboratory, especially if it was caused originally by disruption of weak forces. If the denaturing chemicals are removed, the protein returns to its "native" shape and normal function.

Chaperonins help shape proteins

There are two occasions when a polypeptide chain is in danger of binding the wrong ligand. First, following denaturation, hydrophobic R groups, previously on the inside of the protein away from water, become exposed on the surface. Since these groups can interact with similar groups on other molecules, the denatured proteins may aggregate and become insoluble, losing their function. Second, when a protein has just been synthesized and has not yet folded completely, it could present a surface that binds the wrong molecule.

Living systems limit inappropriate protein interactions by making a class of proteins called, appropriately, chaperonins (recall the chaperones—usually teachers—at school dances who try to prevent "inappropriate interactions" among the students). Chaperonins were first identified in fruit flies as "heat shock" proteins, which prevented denaturing proteins from clumping together when the flies' temperature was raised.

Some chaperonins work by trapping proteins in danger of inappropriate binding inside a molecular "cage" (Figure 3.10). This cage is composed of identical sub units, and is itself a good example of quaternary protein structure. Inside the cage, the targeted protein folds into the right shape, and then is released at the appropriate time and place.

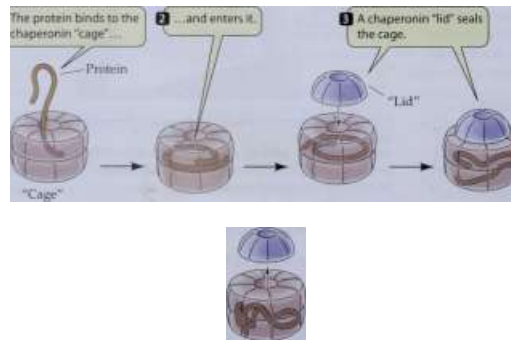
3.10 Chaperonins Protect Proteins from Inappropriate Binding

Chaperonins surround new or denatured proteins and prevent them from binding to the wrong ligand.

f

The protein binds to the chaperonin "cage"...

A chaperonin "lid" seals the cage.



I The protein folds into its appropriate shape and is released.



MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 43

Carbohydrates: Sugars and Sugar Polymers

Carbohydrates are a diverse group of compounds containing primarily carbon atoms flanked by hydrogen and hydroxyl groups ($\text{H}-\text{C}-\text{OH}$). They have two major biochemical roles:

- ▶ They act as a source of energy that can be released in a form usable by body tissues. This energy is stored in strong $\text{C}-\text{C}$ and $\text{C}=\text{O}$ covalent bonds.
- ▶ They serve as carbon skeletons that can be rearranged to form other molecules important for biological structures and functions.

Some carbohydrates are relatively small, with molecular weights less than 100. Others are true macromolecules, with molecular weights in the hundreds of thousands.

There are four categories of biologically important carbohydrates, which we will discuss in turn:

- ▶ Monosaccharides (mono-, "one"; saccharide, "sugar"), such as glucose, ribose, or fructose, are simple sugars and are the monomers out of which the larger forms are constructed.
- ▶ Disaccharides (di-, "two") consist of two monosaccharides.
- ▶ Oligosaccharides (oligo-, "several") have several monosaccharides (3 to 20).
- ▶ Polysaccharides (poly-, "many"), such as starch, glycogen, and cellulose, are large polymers composed of hundreds of thousands of monosaccharide units.

The relative proportions of carbon, hydrogen, and oxygen indicated by the general formula for carbohydrates, CH_2O (i.e., the proportions of these atoms are 1:2:1), apply to monosaccharides. In disaccharides, oligosaccharides, and polysaccharides, these proportions differ slightly from the general formula because two hydrogens and an oxygen are lost during the condensation reactions that form them.

Monosaccharides are simple sugars

Green plants produce monosaccharides through photosynthesis, and animals acquire them directly or indirectly from plants. All living cells contain the monosaccharide glucose. Cells use glucose as an energy source, breaking it down through a series of hydrolysis reactions that release stored energy and produce water and carbon dioxide.

Glucose exists in two forms, the straight chain and the ring; the ring structure predominates in more than 99 percent of circumstances. There are also two forms of the ring structure (α -glucose and β -glucose), which differ only in the placement of the —H and —OH attached to carbon 1 (Figure 3.11). The α and β forms interconvert and exist in equilibrium when dissolved in water.

Different monosaccharides contain different numbers of carbons. (The standard convention for numbering carbons shown in Figure 3.11 is used throughout this book.) Most of the monosaccharides found in living systems belong to the d series of optical isomers (see Chapter 2). But some monosaccharides are structural isomers, which have the same kinds and numbers of atoms, but arranged differently by bonding. For example, the hexoses (hex-, "six"), a group of structural isomers, all have the formula $\text{C}_6\text{H}_{12}\text{O}_6$. Included among the hexoses are glucose, fructose (so named because it was first found in fruits), mannose, and galactose (Figure 3.12).

Pentoses (pent-, "five") are five-carbon sugars. Some pentoses are found primarily in the cell walls of plants. Two pentoses are of particular biological importance: Ribose and deoxyribose form part of the backbones of the nucleic acids RNA and DNA, respectively. These two pentoses are not isomers; rather, one oxygen atom is missing from carbon 2 in deoxyribose (de-, "absent") (see Figure 3.12). As we will see in Chapter 12, the absence of this oxygen atom has important consequences for the functional distinction of RNA and DNA.

Aldehyde group

H—C=O

HO—C—H

H—C—OH

H—C—OH

H—C—OH

H—C—OH

Straight-chain form

The straight-chain form of glucose has an aldehyde group at carbon 1.

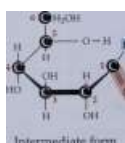


Figure 3.11 Glucose: From One Form to the Other

All glucose molecules have the formula $\text{C}_6\text{H}_{12}\text{O}_5$, but their structures vary. When dissolved in water, the α and β "ring" forms of glucose interconvert. The dark line at

the bottom of each ring indicates that that edge of the molecule extends toward you; the

upper, lighter edge extends back into the page.

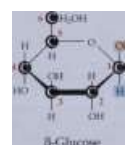


A reaction between this aldehyde group and the hydroxyl group at carbon 5 gives rise to a ring form.



OH

or



Depending on the orientation of the aldehyde group when the ring closes, either of two rapidly and spontaneously interconverting molecules— α -glucose and β -glucose—forms.

Three-carbon sugar

CH_2OH

CH_2

CHO

$\text{H}-\text{C}-\text{OH}$

Glyceraldehyde is the smallest sugar and exists only as the straight-chain form.

Five-carbon sugars

CH_2OH

$\text{H}-\text{C}-\text{OH}$



Ribose and deoxyribose each have five carbons, but very different chemical properties and biological roles.

$\text{OH}-\text{CH}_2$

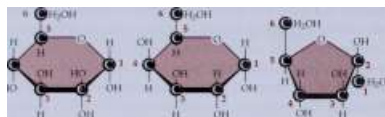
Ribose

Six-carbon sugars

$\text{OH}-\text{CH}_2$

Deoxyribose

$\text{H}-\text{CH}_2$



$\text{HO}-\text{CH}_2$

α -Mannose

α -Galactose

Fructose

These hexoses are isomers. All have the formula $\text{C}_6\text{H}_{12}\text{O}_6$, but each has distinct chemical properties and biological roles.

3.12 Monosaccharides Are Simple Sugars

Monosaccharides are made up of varying numbers of carbons. Some are structural isomers, which have the same number of carbons, but arranged differently. Fructose, for example, is a hexose but forms a five-sided ring like the pentoses.

Glycosidic linkages bond monosaccharides together

Monosaccharides are covalently bonded together by condensation reactions that form glycosidic linkages. Such a linkage between two monosaccharides forms a disaccharide. For example, a molecule of sucrose (table sugar) is formed from a glucose and a fructose molecule, while lactose (milk sugar) contains glucose and galactose.

The disaccharide maltose contains two glucose molecules, but it is not the only disaccharide that can be made from two glucoses. When glucose molecules form glycosidic linkages, the disaccharide product will be one of two types: α -linked or β -linked, depending on whether the molecule that bonds by its carbon 1 is α -glucose or β -glucose (see Figure 3.11). An α linkage with carbon 4 of a second glucose molecule gives maltose, whereas a β linkage gives cellobiose (Figure 3.13).

Maltose and cellobiose are disaccharide isomers, both having the formula $\text{C}_{12}\text{H}_{22}\text{O}_{11}$. However, they are different compounds with different properties. They undergo different chemical reactions and are recognized by different enzymes. For example, maltose can be hydrolyzed to its monosaccharides in the human body, whereas cellobiose cannot. Certain microorganisms have the chemistry to break down cellobiose.

Oligosaccharides contain several monosaccharides linked by glycosidic linkages at various sites. Many oligosaccharides have additional functional groups, which give them special properties. Oligosaccharides are often covalently bonded to proteins and lipids on the outer cell surface, where they serve as cell recognition signals. The human blood groups (such as ABO) get their specificity from oligosaccharide chains.

Maltose is produced when an α -1,4 glycosidic linkage forms between two glucose molecules. The hydroxyl group on carbon 1 of one glucose in the α (down) position reacts with the hydroxyl group on carbon 4 of the other glucose.

$\text{CH}_2\text{OH}-\text{CH}_2\text{OH}$

Formation

α or β α or β , , .

of a linkage

α \rightarrow 4



α

$\text{OH}-\text{HO}-\text{Hf}$

$\text{H}-\text{OH}-\text{H}-\text{OH}$

α -Glucose (3-Glucose

α -1,4 glycosidic linkage $\text{CH}_2\text{OH}-\text{f}-\alpha-\text{CH}_2\text{OH}$

Lfi. $\text{H}-\text{H}'^1$!



$\text{HO}-\text{H}-\text{OH}-\text{H}-\text{OH}$

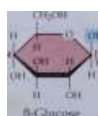
α -Glucose β -Glucose

Maltose

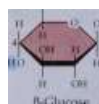
In cellobiose, two glucoses are linked by a β -1,4 glycosidic linkage.

3.73 Disaccharides Are Formed by Glycosidic Linkages

Glycosidic linkages between two monosaccharides create many different disaccharides. Which disaccharide is formed depends on which monosaccharides are linked, and on the site (which carbon atom is linked) and form (α or β) of the linkage.



CH_2OH



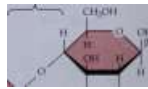
oh Formation β of β linkage

β -1,4 glycosidic linkage

CH_2OH

CH_2OH





H OH

P-Glucose

H OH

p-Glucose

Cellobiose

MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 45

Polysaccharides serve as energy stores or structural materials

Polysaccharides are giant chains of monosaccharides connected by glycosidic linkages. Starch is a polysaccharide of glucose with glycosidic linkages in the α -orientation. Cellulose, too, is a giant polysaccharide made up solely of glucose, but its individual monosaccharides are connected by β linkages (Figure 3.14a). Cellulose is the predominant com-

ponent of plant cell walls, and is by far the most abundant organic compound on Earth. Both starch and cellulose are composed of nothing but glucose, but their very different chemical and physical properties give them distinct biological functions.

Starch can be more or less easily degraded by the actions of chemicals or enzymes. Cellulose, however, is chemically more stable because of its β -glycosidic linkages. Thus starch

(a) Molecular structure

Cellulose

Starch and glycogen



H OH



H H ^ _ o CH,OH

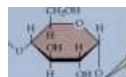
H OH

jL

CH 2 OH

Hydrogen bonding to other cellulose molecules can occur at these points.

CH,OH



H OH O y

r

CH 2 OH CH 2

Branching occurs here.

CH 2 OH

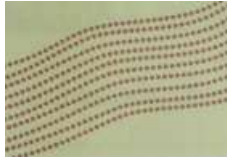


Cellulose is an unbranched polymer of glucose with β -1,4 glycosidic linkages that are chemically very stable.

Glycogen and starch are polymers of glucose with α -1,4 glycosidic linkages, α -1,6 glycosidic linkages produce branching at carbon 6.

(b) Macromolecular structure

Linear (cellulose)



Branched (starch)

N

Highly branched (glycogen)

••«

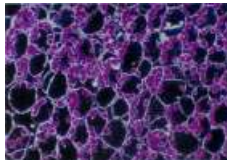


Parallel cellulose molecules hydrogen-bond to form long thin fibrils.

Branching limits the number of hydrogen bonds that can form in starch molecules, making starch less compact than cellulose.

The high amount of branching in glycogen makes its solid deposits less compact than starch.

(c) Polysaccharides in cells



Layers of cellulose fibrils, as seen in this scanning electron micrograph, give plant cell walls great strength.

Dyed red in this micrograph, starch deposits have a large granular shape within cells.

3.14 Representative Polysaccharides

Cellulose, starch, and glycogen demonstrate different levels of branching and compaction in polysaccharides.

Colored pink in this electron micrograph of human liver cells, glycogen deposits have a small granular shape.

46 CHAPTER THREE

(a) Sugar phosphate

Fructose 1,6 biphosphate is involved in the reactions that liberate energy from glucose. (The numbers in its name refer to the carbon sites of phosphate bonding; bis- indicates that two phosphates are present.)

(b) Amino sugars

The monosaccharides glucosamine and galactosamine are amino sugars with an amino group in place of a hydroxyl group.

(c) Chitin

Chitin is a polymer of α -acetylglucosamine; N-acetyl groups provide additional sites for hydrogen bonding between the polymers.

- Phosphate group

$O-P-O-CH_2$

$CH_2-O-P-O-$

I

O-

$H-O-H$

"~" ~^ Fructose OH H

Fructose 1,6 biphosphate

CH_2OH

CH_2OH



[g Amino

group Glucosamine



OH

BBS

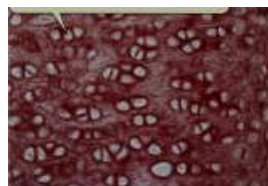
Galactosamine

α -acetyl Glucosamine C=O group

3.75 Chemically Modified Carbohydrates

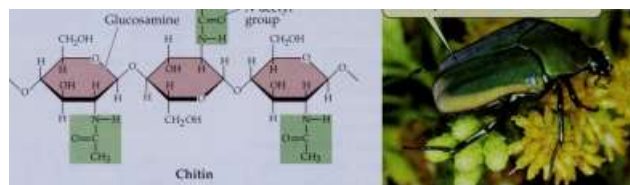
Added functional groups modify the form and properties of a carbohydrate.

Galactosamine is an important component of cartilage, a connective tissue in vertebrates.



The external skeletons of insects are made up of chitin.

CH_2OH



Chitin

is a good storage medium that can be easily broken down to supply glucose for energy-producing reactions, while cellulose is an excellent structural material that can withstand harsh environmental conditions without changing.

Starch actually comprises a large family of giant molecules of broadly similar structure. While all starches are large polymers of glucose with α -linkages, the different starches can be distinguished by the amount of branching that occurs at carbons 1 and 6 (Figure 3.14fr). Some starches are highly branched; others are not. Plant starches, called amylose, are not highly

branched. The polysaccharide glycogen, which stores glucose in animal livers and muscles, is highly branched.

Starch and glycogen serve as energy storage compounds for plants and animals, respectively. These polysaccharides are readily hydrolyzed to glucose monomers, which in turn can be further degraded to liberate and convert their stored energy to forms that can be used for cellular activities. If it is glucose that is actually needed for fuel, why must it be stored as a polymer? The reason is that 1,000 glucose molecules would exert 1,000 times the osmotic pressure (causing water to enter the cells; see Chapter 5) of a single glycogen molecule. If it were not for polysaccharides, many organisms would expend a lot of time and energy expelling excess water.

Chemically modified carbohydrates contain other groups

Some carbohydrates are chemically modified by adding functional groups such as phosphate and amino groups (Figure 3.15). For example, carbon 6 in glucose may be oxidized from $\text{—CH}_2\text{OH}$ to a carboxyl group (—COOH), producing glucuronic acid. Or a phosphate group may be added to one or more of the —OH sites. Some of these sugar phosphates, such as fructose 1,6-bisphosphate, are important intermediates in cellular energy reactions.

When an amino group is substituted for an —OH group, amino sugars, such as glucosamine and galactosamine, are produced. These compounds are important in the extracellular matrix, where they form parts of proteins involved in keeping tissues together. Galactosamine is a major component of cartilage, the material that forms caps on the ends of bones and stiffens the protruding parts of the ears and nose. A derivative of glucosamine produces the polymer chitin, which is the principal structural polysaccharide in the skeletons of insects, crabs, and lobsters, as well as in the cell walls of fungi. Fungi and insects (and their relatives) constitute more than 80 percent of the species ever described, and chitin is one of the most abundant substances on Earth.

Base

Nucleic Acids:

Informational Macromolecules

Base

© ■

Phosphate



Base

Nucleotide

The nucleic acids are linear polymers specialized for the storage, transmission, and use of information. There are two types of nucleic acids: DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). DNA molecules are giant polymers that encode hereditary information and pass it from generation to generation. Through an RNA intermediate, the information encoded in DNA is also used to make specific proteins. RNA molecules of various types copy the information in segments of DNA to specify the sequence of amino acids in proteins. Information flows from DNA to DNA in reproduction, but in the nonreproductive activities of the cell, information flows from DNA to RNA to proteins, which ultimately carry out these functions. What compositions, structures, and properties of nucleic acids permit them to play these fundamental roles in living systems?

The nucleic acids have characteristic properties

Nucleic acids are composed of monomers called nucleotides, each of which consists of a pentose sugar, a phosphate group, and a nitrogen-containing base—either a pyrimidine or a purine (Figure 3.16). (Molecules consisting

The base may be either

a pyrimidine: |

or a purine: I ""N



Ribose or Nucleoside deoxyribose

rn^ 3.76 Nucleotides Have Three Components

A nucleotide consists of a phosphate group, a pentose sugar, and a nitrogen-containing base— all linked together by covalent bonds. The nitrogenous bases fall into two categories: Purines have two fused rings, and the smaller pyrimidines have a single ring.

of a pentose sugar and a nitrogenous base, but no phosphate group, are called nucleosides.) In DNA, the pentose sugar is deoxyribose, which differs from the ribose found in RNA by one oxygen atom (see Figure 3.12).

In both RNA and DNA, the backbone of the molecule consists of alternating pentose sugars and phosphates (sugar—phosphate—sugar—phosphate—). The bases are attached to the sugars and project from the chain (Figure 3.17). The nucleotides are joined by covalent bonds in what are

rn



3.7 7 Distinguishing Characteristics of DNA and RNA

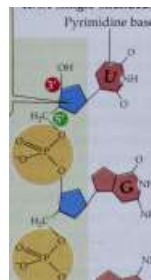
RNA is usually a single strand. DNA usually consists of two strands running in opposite directions.

The numbering of ribose carbons * is the basis for identification of 5' and 3' ends Ribose of DNA and sugar RNA strands.



RNA (single-stranded)

Pyrimidine base

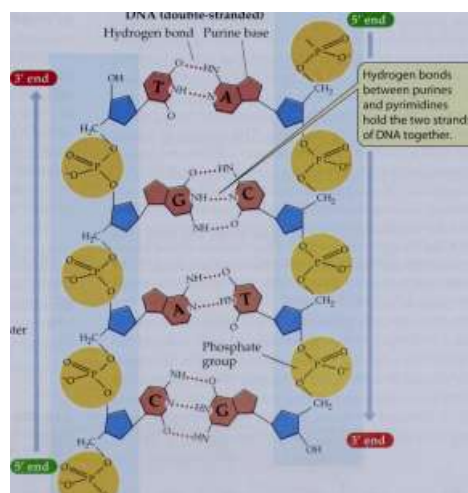


DNA (double-stranded)

Hydrogen bond Purine base

H,C

\



In RNA, the bases are attached to the ribose. The bases in RNA are the purines adenine (A) and guanine (G) and the pyrimidines cytosine (C) and uracil (U).

In DNA, the bases are attached to deoxyribose, and the base thymine (T) is found instead of uracil.

48 CHAPTER THREE

called phosphodiester linkages between the sugar of one nucleotide and the phosphate of the next ("diester" refers to the two bonds formed by —OH groups reacting with acidic phosphate groups). The phosphate groups link carbon 3 in one pentose sugar to carbon 5 in the adjacent sugar.

Most RNA molecules consist of only one polynucleotide chain. DNA, however, is usually double-stranded; it has two polynucleotide chains held together by hydrogen bonding between their nitrogenous bases. The two strands of DNA run in opposite directions. You can see what this means by drawing an arrow through the phosphate group from carbon 5 to carbon 3 in the next ribose. If you do this for both strands, the arrows point in opposite directions. This antiparallel orientation is necessary for the strands to fit together in three-dimensional space.

The uniqueness of a nucleic acid resides in its base sequence

Only four nitrogenous bases—and thus only four nucleotides—are found in DNA. The DNA bases and their abbreviations are adenine (A), cytosine (C), guanine (G), and thymine (T).

A key to understanding the structures and functions of nucleic acids is the principle of complementary base pairing through hydrogen bond formation. In double-stranded DNA, adenine and thymine always pair {AT}, and cytosine and guanine always pair (CG).

Base pairing is complementary because of three factors: the corresponding sites for hydrogen bonding, the geometry of the sugar-phosphate backbone that brings opposite bases near each other, and the molecular sizes of the paired bases. Adenine and guanine are both purines, consisting of two fused rings. Thymine and cytosine are both pyrimidines, consisting of only one ring. The pairing of a large purine with a small pyrimidine ensures a stable and consistent dimension to the double-stranded molecule of DNA.

Ribonucleic acids are also made up of four different monomers, but the nucleotides differ from those of DNA. In RNA the nucleotides are termed ribonucleotides (the ones in DNA are deoxyribonucleotides). They contain ribose rather than deoxyribose, and instead of the base thymine, RNA uses the base uracil (U) (Table 3.3). The other three bases are the same as in DNA.

Although RNA is generally single-stranded, complementary hydrogen bonding between ribonucleotides can take place. These bonds play important roles in determining the shapes of some RNA molecules and in associations between RNA molecules during protein synthesis. During the DNA-directed synthesis of RNA, complementary base pairing also takes place between ribonucleotides and the

J j Distinguishing RNA from DNA

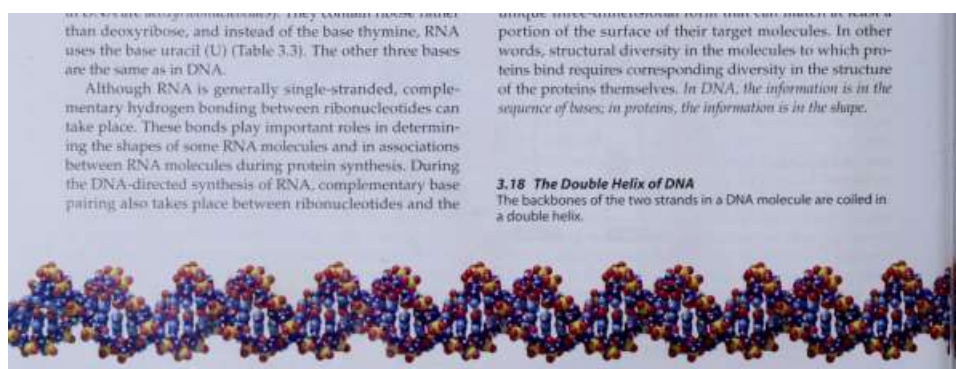
Thymine

bases of DNA. In RNA, guanine and cytosine pair (GC) as in DNA, but adenine pairs with uracil (AU). Adenine in an RNA strand can pair either with uracil (in another RNA strand) or with thymine (in a DNA strand).

The three-dimensional appearance of DNA is strikingly uniform. The segment shown in Figure 3.18 could be from any DNA molecule. Through hydrogen bonding, the two complementary polynucleotide strands pair and twist to form a double helix. When compared with the complex and varied tertiary structures of different proteins, this uniformity is surprising. But this structural contrast makes sense in terms of the functions of these two classes of macro-molecules.

DNA is a purely informational molecule. The information in DNA is encoded in the sequence of bases carried in its strands. Its variations—the different sequences of bases—are "internal." It can be read easily and reliably, in a specific order. A uniformly shaped molecule like DNA can be interpreted by standard molecular machinery, and any cell's machinery can read any molecule of DNA.

Proteins, on the other hand, have good reason to be so varied. In particular, proteins must recognize their own specific "target" molecules. They do this by having a unique three-dimensional form that can match at least a portion of the surface of their target molecules. In other words, structural diversity in the molecules to which proteins bind requires corresponding diversity in the structure of the proteins themselves. In DNA, the information is in the sequence of bases; in proteins, the information is in the shape.



3.18 The Double Helix of DNA
The backbones of the two strands in a DNA molecule are coiled in a double helix.

MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 49

DNA is a guide to evolutionary relationships

Because DNA carries hereditary information between generations, a theoretical series of DNA molecules with changes in base sequences stretches back through evolutionary time. Of course, we cannot study all of these DNA molecules, because many of their organisms have become extinct. However, we can study the DNA of living organisms, which are judged to have changed little through millions of years. Comparisons and contrasts of these DNA molecules can be added to evidence from fossils and other sources to reveal the evolutionary record, as we will see in Chapter 24.

Closely related living species should have more similar base sequences than species judged by other criteria to be more distantly related. Indeed, this is the case. The examination of base sequences confirms many of the evolutionary relationships

that have been inferred from the more traditional study of body structures or studies of biochemistry and physiology. For example, the closest living relative of humans (*Homo sapiens*) is the chimpanzee (genus *Pan*), which shares more than 98 percent of its DNA base sequence with human DNA.

This confirmation of well-established evolutionary relationships gives credibility to the use of DNA to elucidate relationships when studies of structure are not possible or are not conclusive. For example, DNA studies revealed a close evolutionary relationship between starlings and mockingbirds that was not expected on the basis of anatomy or behavior.

DNA studies support the division of the prokaryotes into two domains, Bacteria and Archaea. Each of these two groups of prokaryotes is as distinct from the other as either is from the Eukarya, the third domain into which living things are classified (see Chapter 1). In addition, DNA comparisons support the hypothesis that certain subcellular compartments of eukaryotes (the organelles called mitochondria and chloroplasts) evolved from early bacteria that established a stable and mutually beneficial way of life inside larger cells.

Nucleotides have other important roles in the cell

Nucleotides are more than just the building blocks of nucleic acids. As we will describe in later chapters, there are several nucleotides with other functions:

- ▶ ATP (adenosine triphosphate) acts as an energy transducer in many biochemical reactions (see Chapter 6).
- ▶ GTP (guanosine triphosphate) serves as an energy source, especially in protein synthesis. It also has a role in the transfer of information from the environment to the body tissues (see Chapter 12 and 15).
- ▶ cAMP (cyclic AMP), a special nucleotide in which a bond forms between the sugar and phosphate groups within adenosine monophosphate, is essential in many processes, including the actions of hormones and the transmission of information by the nervous system (see Chapter 15).

Lipids: Water-Insoluble Molecules

Lipids are a chemically diverse group of hydrocarbons. The property they all share is an insolubility in water, which is due to the presence of many nonpolar covalent bonds. As we saw in Chapter 2, nonpolar hydrocarbon molecules preferentially aggregate among themselves, away from water, which is polar. When the nonpolar molecules are sufficiently close together, weak but additive van der Waals forces hold them together. These huge macromolecular aggregations are not polymers in a strict chemical sense, since their units (lipid molecules) are not held together by covalent bonds, as are, for example, amino acids in proteins. But they can be considered polymers of individual lipid units.

In this section, we will describe the different types of lipids. Lipids have a number of roles in living organisms:

- ▶ Fats and oils store energy.
- ▶ Phospholipids play important structural roles in cell membranes.
- ▶ The carotenoids help plants capture light energy.
- ▶ Steroids and modified fatty acids play regulatory roles as hormones and vitamins.
- ▶ The fat in animal bodies serves as thermal insulation.
- ▶ A lipid coating around nerves acts as electrical insulation.
- ▶ Oil or wax on surfaces of skin, fur, and feathers repel water.

Fats and oils store energy

Chemically, fats and oils are triglycerides, also known as simple lipids. Triglycerides that are solid at room temperature (20°C) are called fats; those that are liquid at room temperature are called oils. Triglycerides are composed of two types of building blocks: fatty acids and glycerol. Glycerol is a small molecule with three hydroxyl (—OH) groups. Fatty acids are made up of a long nonpolar hydrocarbon

Allow your eyes to follow the yellow phosphorus atoms and their attached red oxygen atoms in the two helical backbones.

The paired bases are stacked in the center of the coil; concentrate on the lighter blue nitrogen and darker blue carbon atoms.

The small white atoms are hydrogens.

50 CHAPTER THREE

Triglyceride

Glycerol (an alcohol)

Fatty acid molecules

CH 2 -

I OH

OH

o=c

CH-

CH-

I OH

OH

I

o=c

CH,

CH 2

I ' OH

OH

o=c

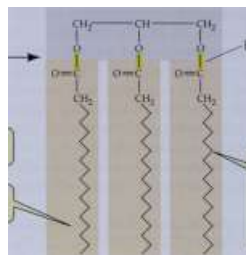
I CH,

3H 2 o

The synthesis of an ester is a condensation reaction.



Nonpolar hydrocarbon chains are hydrophobic.



Ester linkage

Each junction in this lipid tail represents a carbon with hydrogens to fill available covalent bonds:

C C

/\ or /\

H H H H H

3.7 9 Synthesis of a Triglyceride

In living things, the reaction that forms triglycerides is more complex, but the end result is as shown here.

chain and a polar carboxyl functional group ($-\text{COOH}$). A triglyceride contains three fatty acid molecules and one molecule of glycerol (Figure 3.19).

The carboxyl group of a fatty acid can react with the hydroxyl group of glycerol to form an ester (the reaction product of an acid and an alcohol) and water. The three fatty acids in a triglyceride molecule need not all have the same hydrocarbon chain length or structure.

In saturated fatty acids, all the bonds between the carbon atoms in the hydrocarbon chain are single bonds—there are no double bonds. That is, all the bonds are saturated with hydrogen atoms (Figure 3.20a). These fatty acid molecules are relatively rigid and straight, and they pack together tightly, like pencils in a box.

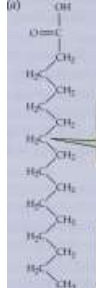
In unsaturated fatty acids, the hydrocarbon chain contains one or more double bonds. Oleic acid, for example, is a monounsaturated fatty acid that has one double bond near the middle of the hydrocarbon chain, which causes a kink in the molecule (Figure 3.20b). Some fatty acids have more than one double bond—are polyunsaturated—and have multiple kinks.

These kinks prevent the molecules from packing together tightly.

The kinks are important in determining the fluidity and melting point of a lipid. Animal fats are usually solids at room temperature, and their triglycerides tend to have many long-chain saturated fatty acids, packed well together. The triglycerides of plants, such as corn oil, tend to have short or unsaturated fatty acids. Because of their kinks, these fatty acids pack poorly together, and these triglycerides are usually liquids at room temperature.

3.20 Saturated and Unsaturated Fatty Acids

(a) In saturated fatty acids, the straight chain allows the molecule to pack tightly among other similar molecules. (b) In unsaturated fatty acids, kinks in the chain prevent close packing.



ch 3 Palmitic acid

(b) OH

O=C

All bonds between carbon atoms are single, making a saturated fatty acid.

The straight chain allows this molecule to pack tightly among other similar molecules.



CH₃

CH₂ : CH₂ CH₂

HC

HC

/

\

/'

CH₂

CH₂

CH₂

A double bond between two carbons makes an unsaturated fatty acid. P[^]

CH₂ HC

HC

The kinks prevent close packing.



CH_2CH_2

CH_2CH_2



Linoleic acid

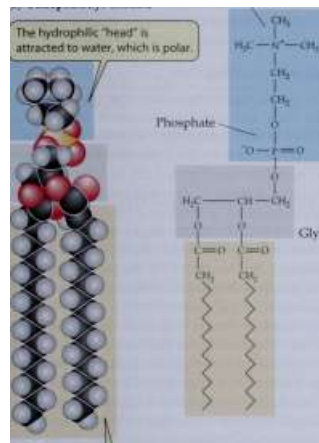
.CH₃

MACROMOLECULES: THEIR CHEMISTRY AND BIOLOGY 51

(a) Phosphatidyl choline

Choline

The hydrophilic "head" is attracted to water, which is polar.



Glycerol

The hydrophobic "tails" are not attracted to water.

(b) Membrane phospholipid

() ~~~~ Hydrophilic head ||> Hydrophobic tails

Throughout this book, phospholipids in membranes are shown with this symbol.

3.21 Phospholipid Structure

(a) Phosphatidyl choline (lecithin) demonstrates the structure of a phospholipid molecule. In other phospholipids, the amino acid serine, the sugar alcohol inositol, or other compounds replace choline, (b) This generalized symbol is used in this book to represent a membrane phospholipid (see Figure 3.22).

Fats and oils are marvelous storehouses for energy. By taking in excess food, many animal species deposit fat droplets in their cells as a means of storing energy. Some plant species, such as olives, avocados, sesame, castor beans, and all nuts, have substantial amounts of lipids in their seeds or fruits that serve as energy reserves for the next generation. This energy can be tapped by people who eat these plant oils or use them for fuel. Indeed, the famous German engineer Rudolf Diesel used peanut oil to power one of his early automobile engines in 1900. As petroleum stores become depleted, there is some interest in using plant oils commercially as fuel.

Phospholipids form the core of biological membranes

Because lipids and water do not interact, a mixture of water and lipids forms two distinct phases. Many biologically important substances—such as ions, sugars, and free amino acids—that are soluble in water are insoluble in lipids.

Like triglycerides, phospholipids contain fatty acids bound to glycerol by ester linkages. In phospholipids, however, any one of several phosphate-containing compounds replaces one of the fatty acids (Figure 3.21). The phosphate functional group has a negative electric charge, so this portion of the molecule is hydrophilic, attracting polar water molecules. But the two fatty acids are hydrophobic, so they aggregate away from water.

In an aqueous environment, phospholipids line up in such a way that the nonpolar, hydrophobic "tails" pack tightly together, and the phosphate-containing "heads" face outward, where they interact with water. The phospholipids thus form a bilayer, a sheet two molecules thick, from which water is excluded (Figure 3.22). Biological membranes have this kind of lipid bilayer structure, and we will devote all of Chapter 5 to their biological functions.

Because the word "lipid" defines compounds in terms of their solubility rather than their structural similarity, a great variety of different chemical structures are included as lipids.

Carotenoids and steroids

The next two lipid classes we'll discuss—the carotenoids and the steroids—have chemical structures very different from those of triglycerides and phospholipids and from each other. Both carotenoids and steroids are synthesized by covalent linking and chemical modification of isoprene to form a series of isoprene units:

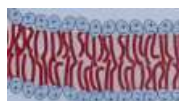
CH₂,

CH₂=C—CH=CH₂

carotenoids trap light energy. The carotenoids are a

family of light-absorbing pigments found in plants and animals. Beta-carotene ((3-carotene) is one of the pigments that traps light energy in leaves during photosynthesis. In humans, a molecule of (3-carotene can be broken down into

Water



Water

Hydrophilic "heads"

Hydrophobic fatty acid "tails"

Hydrophilic "heads"

Phospholipid bilayer

3.22 Phospholipids Form a Bilayer

In an aqueous environment, hydrophobic interactions bring the "tails" of phospholipids together in the interior of a phospholipid bilayer. The hydrophilic "heads" face outward on both sides of the bilayer, where they interact with the surrounding water molecules.

52 CHAPTER THREE

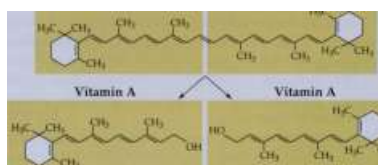
<■ ■ ~ > ,

Splitting p-carotene in the middle

produces two vitamin A molecules.

(3-Carotene

H.C



H₂C CH₂,

3.23 (3-Carotene is the Source of Vitamin A

The carotenoid p-carotene is symmetrical around its central double bond; when split, p-carotene becomes two vitamin A molecules. The simplified structural formula used here is standard chemical shorthand for large organic molecules with many carbon atoms. Structural formulas are simplified by omitting the C's (for carbon atoms) at the intersections of the lines representing covalent bonds. H's to fill all the available bonding sites on each C are assumed.

two vitamin A molecules (Figure 3.23), from which we make the pigment rhodopsin, which is required for vision. Carotenoids are responsible for the colors of carrots, tomatoes, pumpkins, egg yolks, and butter.

steroids are signal molecules. The steroids are a family of organic compounds whose multiple rings share carbons (Figure 3.24). The steroid cholesterol is an important constituent of membranes. Other steroids function as hormones, chemical signals that carry messages from one part of the body to another. Testosterone and the estrogens are steroid hormones that regulate sexual development in vertebrates. Cortisol and related hormones play many regulatory roles in the digestion of carbohydrates and proteins, in the maintenance of salt balance and water balance, and in sexual development.

Cholesterol is synthesized in the liver and is the starting material for making testosterone and other steroid hormones, as well as the bile salts that help break down dietary fats so that they can be digested. Cholesterol is absorbed from foods such as milk, butter, and animal fats. An

excess of cholesterol in the blood can lead to its deposition (along with other substances) in the arteries, a condition that may lead to arteriosclerosis and heart attack.

Some lipids are vitamins

Vitamins are small organic molecules that are not synthesized in the body and must be acquired from dietary sources, and whose deficiencies lead to defined diseases.

Vitamin A is formed from the P-carotene found in green and yellow vegetables (see Figure 3.23). In humans, a deficiency of vitamin A leads to dry skin, eyes, and internal body surfaces; retarded growth and development; and night blindness, which is a diagnostic symptom for the deficiency. Vitamin D regulates the absorption of calcium from the intestines. It is necessary for the proper deposition of calcium in bones; a deficiency of vitamin D can lead to rickets, a bone-softening disease.

Vitamin E seems to protect cells from damaging effects of oxidation-reduction reactions. For example, it has an important role in preventing unhealthy changes in the double bonds in the unsaturated fatty acids of membrane phospholipids. Commercially, vitamin E is added to some foods to slow spoilage. Vitamin K is found in green leafy plants and is also synthesized by bacteria normally present in the human intestine. This vitamin is essential to the formation of blood clots. Predictably, a deficiency of vitamin K leads to slower clot formation and potentially fatal bleeding from a wound.

Wax coatings repel water

The sheen on human hair is not there only for cosmetic purposes. Glands in the skin secrete a waxy coating that repels water and keeps the hair pliable. Birds that live near water have a similar waxy coating on their feathers. The shiny leaves of holly plants, familiar during winter holidays, also have a waxy coating. Finally, bees make their honeycombs out of wax. All waxes have the same basic structure: They are formed by an ester linkage between a saturated, long-chain fatty acid and a saturated, long-chain

3.24 All Steroids Have the Same Ring Structure

The steroids shown, all important in vertebrates, are composed of carbon and hydrogen and are highly hydrophobic. However, small chemical variations, such as the presence or absence of a methyl or hydroxyl group, can produce enormous functional differences.

CH₃

HO



C\ CH₂ OH

\ / C

OH

H₃C

OH



Cholesterol is a constituent of membranes and is the source of steroid hormones.

Vitamin D₂ can be produced in the skin by the action of light on a cholesterol derivative.

Cortisol is a hormone secreted by the adrenal glands.

Testosterone is a male sex hormone.

alcohol. The result is a very long molecule, with 40-60 CH₂ groups. For example, here is the structure of beeswax:

O

II

CH₃-(CH₂)₁₄-C(=O)-CH₂-(CH₂)₂₈-CH₂-OH

28

Fatty acid

y

Alcohol

This highly nonpolar structure accounts for the impermeability of wax to water.

The Interactions of Macromolecules

We have treated the classes of macromolecules as if each were separate from the others. In cells, however, certain macromolecules of different classes may be covalently bonded to one another. Proteins with attached oligosaccharides are called glycoproteins (glyco-, "sugar"). The specific oligosaccharide chain attached can determine where within the cell a newly synthesized protein will reside. Other carbohydrate chains covalently bond to lipids, resulting in glycolipids, which reside in the cell surface membrane, with the carbohydrate chain extending out into the cell's environment. The carbohydrates that determine a person's blood type (A, B, AB, or O) are attached to either proteins or lipids sticking out from the surfaces of red blood cells.

We have already mentioned the fact that proteins can bind noncovalently to other proteins in quaternary structures. But proteins can bind noncovalently to the other types of macromolecules as well. For example, there are hundreds of different proteins that recognize and bind to DNA, regulating its function. Other proteins, in combination with cholesterol and other lipids, form lipoproteins. Some lipoproteins serve as carrier proteins, which make it possible to move very hydrophobic lipids such as cholesterol through water-rich environments such as the blood.

Summary

Macromolecules: Giant Polymers

► Macromolecules are constructed by the formation of covalent bonds between smaller molecules called monomers. Macromolecules include polysaccharides, proteins, and nucleic acids. Review Figure 3.1 and Table 3.1

► Macromolecules have specific, characteristic three-dimensional shapes that depend on the structures, properties, and sequence of their monomers. Different functional groups give local sites on macromolecules specific properties that are important for their biological functioning and their interactions with other macromolecules.

Condensation Reactions

► Monomers are joined by condensation reactions, which release a molecule of water for each bond formed. Hydrolysis reactions use water to break polymers into monomers. Review Figure 3.2

Proteins: Polymers of Amino Acids

► The functions of proteins include support, protection, catalysis, transport, defense, regulation, and movement. Protein function sometimes requires an attached prosthetic group.

► There are 20 amino acids found in proteins. Each amino acid consists of an amino group, a carboxyl group, a hydrogen, and a side chain bonded to the α carbon atom. Review Table 3.2

► The side chains of amino acids may be charged, polar, or hydrophobic; there are also "special cases," such as the —SH groups, which can form disulfide bridges. The side chains give different properties to each of the amino acids. Review Table 3.2 and Figure 3.3

► Amino acids are covalently bonded together by peptide linkages, which form by condensation reactions between the carboxyl and amino groups. Review Figure 3.4

► The polypeptide chains of proteins are folded into specific three-dimensional shapes. Four levels of structure are possible: primary, secondary, tertiary, and quaternary.

► The primary structure of a protein is the sequence of amino acids bonded by peptide linkages. This primary structure determines both the higher levels of structure and protein function. Review Figure 3.5a

► Secondary structures of proteins, such as α helices and

β (3) pleated sheets, are maintained by hydrogen bonds between atoms of the amino acid residues. Review Figure 3.5b,c

- ▶ The tertiary structure of a protein is generated by bending and folding of the polypeptide chain. Review Figures 3.5d, 3.6
- ▶ The quaternary structure of a protein is the arrangement of polypeptides in a single functional unit consisting of more than one polypeptide subunit. Review Figures 3.5e, 3.7
- ▶ Weak chemical interactions are important in the binding of proteins to other molecules. Review Figure 3.8
- ▶ Proteins denatured by heat, acid, or certain chemicals lose their tertiary and secondary structure as well as their biological function. Renaturation is not always possible. Review Figure 3.9
- ▶ Chaperonins assist protein folding by preventing binding to inappropriate ligands. Review Figure 3.10

Carbohydrates: Sugars and Sugar Polymers

- ▶ All carbohydrates contain carbon bonded to H and OH groups.
- ▶ Hexoses are monosaccharides that contain six carbon atoms. Examples of hexoses include glucose, galactose, and fructose, which can exist as chains or rings. Review Figures 3.11, 3.12
- ▶ The pentoses are five-carbon monosaccharides. Two pentoses, ribose and deoxyribose, are components of the nucleic acids RNA and DNA, respectively. Review Figure 3.12
- ▶ Glycosidic linkages may have either a or (3 orientation in space. They covalently link monosaccharides into larger units such as disaccharides (for example, cellobiose), oligosaccharides, and polysaccharides. Review Figures 3.13, 3.14
- ▶ Cellulose, a very stable glucose polymer, is the principal component of the cell walls of plants. It is formed by glucose units linked together by (3-glycosidic linkages between carbons 1 and 4. Review Figure 3.14
- ▶ Starches, less dense and less stable than cellulose, store energy in plants. Starches are formed by (x-glycosidic linkages between carbons 1 and 4 and are distinguished by the amount of branching that occurs through glycosidic bond formation at carbon 6. Review Figure 3.14
- ▶ Glycogen contains a-1,4 glycosidic linkages and is highly branched. Glycogen stores energy in animal livers and muscles. Review Figure 3.14
- ▶ Chemically modified monosaccharides include the sugar phosphates and amino sugars. A derivative of the amino sugar glucosamine polymerizes to form the polysaccharide

54 CHAPTER THREE

chitin, which is found in the cell walls of fungi and the exoskeletons of insects. Review Figure 3.15

Nucleic Acids: Informational Macromolecules

- ▶ In cells, DNA is the hereditary material. Both DNA and RNA play roles in the formation of proteins. Information flows from DNA to RNA to protein.
- ▶ Nucleic acids are polymers made up of nucleotides. Nucleotides consist of a phosphate group, a sugar (ribose in RNA and deoxyribose in DNA), and a nitrogen-containing base. In DNA the bases are adenine, guanine, cytosine, and thymine, but in RNA uracil substitutes for thymine. Review Figure 3.16 and Table 3.3
- ▶ In the nucleic acids, the bases extend from a sugar-phosphate backbone. The information content of DNA and RNA resides in their base sequences.
- ▶ RNA is single-stranded. DNA is a double-stranded helix in which there is complementary, hydrogen-bonded base pairing between adenine and thymine (AT) and guanine and cytosine (GC). The two strands of the DNA double helix run in opposite directions. Review Figures 3.17, 3.18
- ▶ Comparing the DNA base sequences of different living species provides information on their evolutionary related-ness.

Lipids: Water-Insoluble Molecules

- ▶ Although lipids can form gigantic structures, such as lipid droplets and membranes, these aggregations are not chemically macromolecules because the individual units are not linked by covalent bonds.
- ▶ Fats and oils are composed of three fatty acids covalently bonded to a glycerol molecule by ester linkages. Review Figure 3.19
- ▶ Saturated fatty acids have a hydrocarbon chain with no double bonds. The hydrocarbon chains of unsaturated fatty acids have one or more double bonds that bend the chain, making close packing less possible. Review Figure 3.20
- ▶ Phospholipids have a hydrophobic hydrocarbon "tail" and a hydrophilic phosphate "head." Review Figure 3.21
- ▶ In water, the interactions of the hydrophobic tails and hydrophilic heads generate a phospholipid bilayer that is two molecules thick. The head groups are directed outward, where they interact with the surrounding water. The tails are packed

together in the interior of the bilayer. Review Figure 3.22

- ▶ Carotenoids trap light energy in green plants. (β-Carotene can be split to form vitamin A, a lipid vitamin. Review Figure 3.23)
- ▶ Some steroids, such as testosterone, function as hormones. Cholesterol is synthesized by the liver and has a role in some cell membranes, as well as in the digestion of other fats. Too much cholesterol in the diet can lead to arteriosclerosis. Review Figure 3.24
- ▶ Vitamins are substances that are required for normal functioning but that must be acquired from the diet.

The Interactions of Macromolecules

- ▶ Both covalent and noncovalent linkages are found between the various classes of macromolecules.
- ▶ Glycoproteins contain an oligosaccharide "label" that directs the protein to the proper cell destination. The carbohydrate groups of glycolipids are displayed on the cell's outer surface, where they serve as recognition signals.
- ▶ Hydrophobic interactions bind cholesterol to the protein that transports it in the blood.

For Discussion

1. Phospholipids make up a major part of every biological membrane; cellulose is the major constituent of the cell walls of plants. How do the chemical structures and physical properties of phospholipids and cellulose relate to their functions in cells?
2. Suppose that, in a given protein, one lysine is replaced by aspartic acid (see Table 3.2). Does this change occur in the primary structure or in the secondary structure? How might it result in a change in tertiary structure? In quaternary structure?
3. If there are 20 different amino acids commonly found in proteins, how many different dipeptides are there? How many different tripeptides? How many different trinucleotides? How many different single-stranded RNAs composed of 200 nucleotides?
4. Contrast the following three structures: hemoglobin, a DNA molecule, and a protein that spans a biological membrane.

Self-quizzes and Supplemental Readings for each chapter are on the Student Web Site/CD-ROM.



The Organization of Cells

#Jane, a resident of a nursing home, enjoyed visits from her grandchildren. They often brought her gifts, but on this winter day, an unprotected sneeze from one of them led to her catching a cold. Over the next week, she stayed in bed while her body fought off this infection. But while her body was weakened by the cold, a more dangerous event occurred.

Tiny bacteria called *Streptococcus pneumoniae* had been living in Jane's throat for several months. Now they began to multiply, invading healthy tissues in her lungs. The lung tissues reacted by swelling—the hallmark of pneumonia. Jane's doctor arrived to find her with shaking chills, chest pain, and a cough that produced a greenish ooze. After capturing some of the green material in a test tube, the physician sent it to a laboratory where the bacteria were clearly seen and a diagnosis of bacterial pneumonia was confirmed. The doctor prescribed antibiotics, which killed the bacteria, and Jane recovered within a week.

Both the bacteria that caused Jane's pneumonia and the lung tissues they invaded are made up of cells, the units of biological structure and function. Bacteria and lung cells are very different in size and structure. The fundamental differences between them allowed the antibiotic to kill the bacteria while sparing the lung cells. Nevertheless, both bacteria and lung cells perform similar biological functions, taking in substances from their environment and refashioning them for their own uses.

The next four chapters are about how cells perform their basic functions. We begin by describing the structural features of simple cells, such as bacteria, and more complex ones, like lung cells. We will discuss cell sizes and shapes, and we will describe the surface structures and internal compartments that permit cells to transform energy, move, change shape, communicate, and maintain internal conditions that are different from their immediate surroundings.

The Cell: The Basic Unit of Life

All organisms are composed of cells. All cells come from preexisting cells. These two statements constitute the cell theory. Just as atoms are the units of chemistry, cells are the building blocks of life. They are composed of water molecules and the small and large molecules we examined in the pre-

Units of Life, Agents of Disease

Streptococcus pneumoniae are bacterial cells—units of biological structure and function and, in the human lung, a cause of disease.

vious two chapters. Each cell contains at least 10,000 different types of molecules, most of them present in many copies. Cells use these molecules to transform matter and energy, to respond to their environment, and to reproduce themselves.

The cell theory has two important implications. First, it means that studying cell biology is in some sense the same as studying life. The principles that underlie the functions of the single cell in a bacterium are similar to those governing the 60 trillion cells in your body. Second, it means that life is continuous. All those cells in your body came from a single cell, the fertilized egg, which came from the fusion of two cells, a sperm and an egg from your parents, whose cells came from their fertilized eggs, and so on.

Cell size is limited by the surface area-to-volume ratio

Most cells are tiny. The volume of cells ranges from 1 to 1,000 urn 3 (Figure 4.1). The eggs of some birds are enormous exceptions, to be sure, and individual cells of several types of algae and bacteria are large enough to be viewed with the unaided eye. And although neurons (nerve cells) have a volume that is within the "normal" cell range, they often have fine projections that may extend for meters, carrying signals from one part of a large animal to another. But by and large, cells are minuscule. The reason for this relates to the change in the surface area-to-volume ratio (SA/V) of any object as it increases in size.



56 CHAPTER FOUR

This scale is logarithmic. Each unit is ten times bigger than the previous unit.

7 ^

0.1 nni Iran 10 nm 100 nm 1 urn 10 urn 100 urn 1mm 1cm 0.1m lm 10 m

Jll I I 1,1 II 1,11 _l .1. I I III 11 I 1 I I I I'II I I I I III I | I I I I I I Nil I II I I I Mil I

100 m 1 km

CrtJ

V

t

c

Unaided eye

Light microscope

Atoms

Lipids

Electron microscope

T2 phage

& &

CT^ Protein

Small molecules



s

Chloroplast /)

o°°o Most bacteria

Plant

nCfflyft animal %& cells Fish

e §8 Hummingbird



A

Most cell diameters are in the range of 1 -100 urn.



Giant

redwood

tree

Human

fm^ 4.1 The Scale of Life

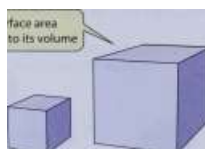
The scale shows the relative sizes of molecules, cells, and multicellular organisms. The measurements are defined in the table on the inside front cover.



As a cell increases in volume, its surface area also increases, but not to the same extent (Figure 4.2). This phenomenon has great biological significance because the volume of a cell determines the amount of chemical activity it carries out per unit of time, but the surface area determines the amount of substances the cell can take in from the outside environment and the amount of waste products it can release to the environment. As the living cell grows, its rate of waste production and its need for resources increase faster than the surface area. This explains why large organisms must consist of many small cells: Cells are small in volume in order to maintain a large surface area-to-volume ratio.

Smaller surface area compared to its volume

Larger surface area compared to its volume



4.2 Why Cells Are Small

As an object grows, its volume increases more rapidly than its surface area. Cells must maintain a large surface area-to-volume ratio in order to function, which explains why large organisms must be composed of many small cells rather than a few huge ones.

In a multicellular organism, the large surface area represented by the multitude of small cells that make up the whole organism enables the multitude of functions required for survival. Special structures transport food, oxygen, and waste materials to and from the small cells that are distant from the external surface of the organism.

Microscopes are needed to visualize cells

Cells are usually invisible to the human eye. The smallest object a person can typically discern is about 0.2 mm (200 μm) in size. We refer to this measure as resolution, the distance apart two objects must be in order for them to be distinguished as separate; if they are closer together, they appear as a single blur. Many cells are much smaller than 200 μm . Microscopes are used to improve resolution so that cells and their internal structures can be seen.

There are two basic types of microscopes: light microscopes and electron microscopes. The light microscope (LM) uses glass lenses and visible light to form a magnified image of an object. In its contemporary form, the light microscope has a resolving power of about 0.2 μm , which is 1,000 times that of the human eye. This allows visualization of cell sizes and shapes and some internal cell structures. The latter are hard to see under ordinary light, so cells are often killed and stained with dyes to make the structures stand out.

An electron microscope (EM) uses powerful magnets to focus an electron beam, much as the light microscope employs glass lenses to focus a beam of light. Since we cannot see electrons, the electron microscope directs them at a fluorescent screen or a photographic film to create a visible image. The resolving power of electron microscopes is about 0.5 nm, which is 250,000 times finer than that of the human eye. This permits the details of many subcellular structures to be distinguished.

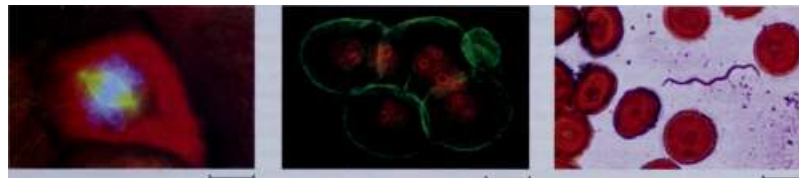
In bright-field microscopy, light passes directly through the cells. Unless natural pigments are present, there is little contrast and details are not distinguished.

25 μm

In phase-contrast microscopy, contrast in the image is increased by emphasizing differences in refractive index (the capacity to bend light), thereby enhancing light and dark regions in the cell.

25 μm

Differential interference-contrast microscopy (Nomarski optics) uses two beams of polarized light. The combined images look as if the cell is casting a shadow on one side.



40 μm

In fluorescence microscopy, a natural substance in the cell or a fluorescent dye that binds to a specific cell material is stimulated by a beam of light, and the longer-wavelength fluorescent light is observed coming directly from the dye.

40 μm

Confocal microscopy uses fluorescent materials but adds a system of focusing both the stimulating and emitted light so that a single plane through the cell is seen. The result is a sharper two-dimensional image than with standard fluorescent microscopy.

75 μm

In stained bright-field microscopy, a stain added to preserved cells enhances contrast and reveals details not otherwise visible. Stains differ greatly in their chemistry and their capacity to bind to cell materials, so many choices are available.



8.5 μm

In transmission electron microscopy (TEM), a beam of electrons is focused on the object by magnets. Objects appear darker if they absorb the electrons. If the electrons pass through, they are detected on a fluorescent screen.



5 μm

Scanning electron microscopy (SEM) directs electrons to the surface of the sample, where they cause other electrons to be

emitted. These electrons are viewed on a screen. The three-dimensional surface of the object can be visualized.

Cryo electron microscopy uses quickly frozen samples to reduce aberrations that are seen when samples are treated chemically. Computer analyses of thick sections can reconstruct a sample in three dimensions.



4.3 Looking at Cells

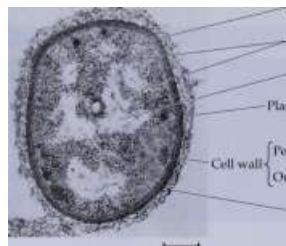
The top six panels represent some of the techniques used in light microscopy. The attributes of light are manipulated in various ways to enhance the images, and many types of stains, dyes, and reagents are used to visualize different cellular components in color. The lower three images were created using electron microscopes, which produce images in black-and-white. Artificial coloration is sometimes added to such micrographs, as in Figure 4.9b.

Many techniques have been developed to enhance the view of cells under both the light and the electron microscope (Figure 4.3). For example, specific dyes will form a colored complex with specific types of molecules (e.g., proteins or DNA), allowing the general composition of various cell structures to be estimated. There are even reagents that will bind to a specific molecule, such as a particular protein, allowing its distribution in the cell to be ascertained.

CHAPTER FOUR

4.4 A Prokaryotic Cell

The bacterium *Pseudomonas aeruginosa* illustrates typical prokaryotic cell structures. The electron micrograph on the left is magnified about 80,000 times. Note the existence of several protective structures external to the plasma membrane.



Cytoplasm

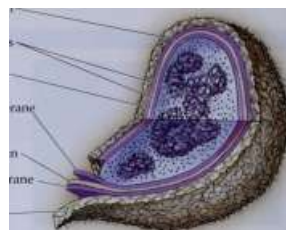
Ribosomes

Nucleoid

Plasma membrane

Peptidoglycan Outer membrane

Capsule



1 urn

All cells are surrounded by a plasma membrane

A plasma membrane separates each cell from its environment, creating a segregated (but not isolated) compartment. The plasma membrane is composed of a phospholipid bi-layer, with the hydrophilic ends of the lipids facing the cell's aqueous interior on one side and the extracellular environment on the other (see Figure 3.22). Proteins are embedded in the lipids. In many cases, the proteins protrude into the cytoplasm and into the extracellular environment. We will devote most of the next chapter to the structure and functions of the plasma membrane, but summarize its roles here:

- ▶ The plasma membrane acts as a selectively permeable barrier, preventing some substances from crossing while permitting other substances to enter and leave the cell.
- ▶ As the cell's boundary with the outside environment, the plasma membrane is important in communicating with adjacent cells and receiving extracellular signals. We will describe this function in Chapter 15.
- ▶ The plasma membrane allows the cell to maintain a more or less constant internal environment. A self-maintaining, constant internal environment is a key characteristic of life and will be discussed in detail in Chapter 40.

Cells show two organizational patterns

Once the microscope was applied to biological samples, it soon became apparent that there are two types of cell structures in the living world.

Prokaryotic cell organization is characteristic of the domain Bacteria and Archaea. Organisms in these domains are called prokaryotes. Their cells do not have membrane-enclosed internal compartments.

Eukaryotic cell organization is found in the domain Eukarya, which includes the protists, plants, fungi, and animals. The genetic material (DNA) of eukaryotic cells is contained in a special membrane-enclosed compartment called the nucleus. Eukaryotic cells also contain other membrane-enclosed compartments in which specific chemical reactions take place. Organisms with this type of cell are known as eukaryotes.

Both prokaryotes and eukaryotes have prospered for many hundreds of millions of years of evolution, and both are great success stories. Let's look first at prokaryotic cells.

Prokaryotic Cells

Prokaryotes can live off more different and diverse energy sources than any other living creatures, and they inhabit greater environmental extremes, such as very hot springs and very salty water. The vast diversity within the prokaryotic domains is the subject of Chapter 26.

Prokaryotic cells are generally smaller than eukaryotic cells, ranging from $0.25 \times 1.2 \mu\text{m}$ to $1.5 \times 4 \mu\text{m}$. So they are generally visible by light microscopy, although their substructures are visible only by electron microscopy. Each prokaryote is a single cell, but many types of prokaryotes are usually seen in chains, small clusters, or even clusters containing hundreds of individuals.

In this section, we will first consider the features that cells in the domains Bacteria and Archaea have in common. Then we will examine structural features that are found in some, but not all, prokaryotes.

All prokaryotic cells share certain features

All prokaryotic cells have the same basic structure (Figure 4.4):

- ▶ The plasma membrane encloses the cell, regulating the traffic of materials into and out of the cell and separating it from its environment.
- ▶ A region called the nucleoid contains the hereditary material (DNA) of the cell.

The rest of the material enclosed in the plasma membrane is called the cytoplasm. Cytoplasm is composed of two parts: the liquid cytosol, and insoluble suspended particles, including ribosomes.

- ▶ The cytosol consists mostly of water that contains dissolved ions, small molecules, and soluble macromolecules such as proteins.
- ▶ Ribosomes are granules about 25 nm in diameter that are sites of protein synthesis.

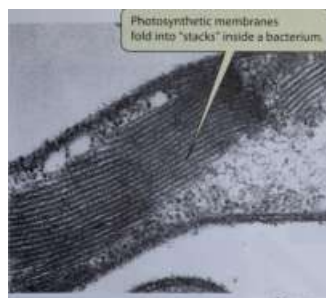
Although structurally less complicated than eukaryotic cells, prokaryotic cells are functionally complex, carrying out thousands of biochemical transformations.

Some prokaryotic cells have specialized features

Many prokaryotic cells have at least a few structural complexities. For example, most prokaryotes have a cell wall

Photosynthetic membranes

fold into "stacks" inside a bacterium.



1 μm

4.5 Some Prokaryotes Have Internal Membrane Systems

The presence of internal membranes contradicts the notion that prokaryotes are nothing more than tiny bags of molecules. These photosynthetic membranes contain compounds needed for photosynthesis.

cated outside the plasma membrane (see Figure 4.4). The rigidity of the cell wall supports the cell and determines its shape. The cell walls of most bacteria, but not archaea, contain peptidoglycan, a polymer of amino sugars, cross-linked by covalent bonds to form a single giant molecule around the entire cell. In some bacteria, another layer—the outer membrane (a polysaccharide-rich phospholipid membrane)—encloses the cell wall. Unlike the plasma membrane, this outer membrane is not a major permeability barrier, and some of its polysaccharides are disease-causing toxins.

Enclosing the cell wall and outer membrane in some bacteria is a layer of slime, composed mostly of polysaccharides and referred to as a capsule. The capsules of some bacteria may protect them from attack by white blood cells in the animals they infect. The capsule helps keep the cell from drying out, and sometimes it traps other cells for the bacterium to attack. Many prokaryotes produce no capsule, and those that do have capsules can survive even if they lose them, so the capsule is not essential to cell life.

THE ORGANIZATION OF CELLS 59

Some groups of bacteria—the cyanobacteria and some others—carry on photosynthesis. In photosynthesis, the energy of sunlight is converted to chemical energy that can be used for a variety of energy-requiring reactions, such as the synthesis of cellular proteins and DNA. In these photosynthetic bacteria, the plasma membrane folds into the cytoplasm to form an internal membrane system that contains bacterial chlorophyll and other compounds needed for photosynthesis (Figure 4.5).

Other groups of prokaryotes possess different types of membranous structures called mesosomes, which may function in cell division or in various energy-releasing reactions. Like the photosynthetic membrane systems, mesosomes are formed by infolding of the plasma membrane. They remain attached to the plasma membrane and never form the free-floating, separate membranous organelles that are characteristic of eukaryotic cells.

Some prokaryotes swim by using appendages called flagella (Figure 4.6a). A single flagellum, made of a protein called flagellin, looks at times like a tiny corkscrew. It spins on its axis like a propeller, driving the cell along. Ring structures anchor the flagellum to the plasma membrane and, in some bacteria, to the outer membrane of the cell wall (Figure 4.6b). We know that the flagella cause the motion of the cell because if they are removed, the cell cannot move.

Pili project from the surface of some groups of bacteria (Figure 4.6c). Shorter than flagella, these threadlike structures help bacteria adhere to one another during mating, as well as to animal cells for protection and food.

Eukaryotic Cells

Animals, plants, fungi, and protists have cells that are usually larger and structurally more complex than those of the prokaryotes (Figure 4.7). To get a sense of the most promi-

4.6 Prokaryotic Projections

Surface projections such as these bacterial flagella (a, b) and pili (c) contribute to movement, to adhesion, and to the complexity of prokaryotic cells.

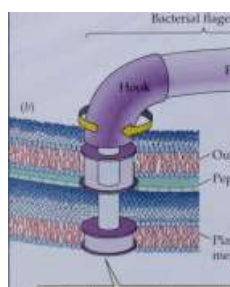
(a)

(c)

Bacterial flagellum .a

A.

Bacterial flagella rotate for locomotion.



Filament

Outer membrane Peptidoglycan

Plasma membrane

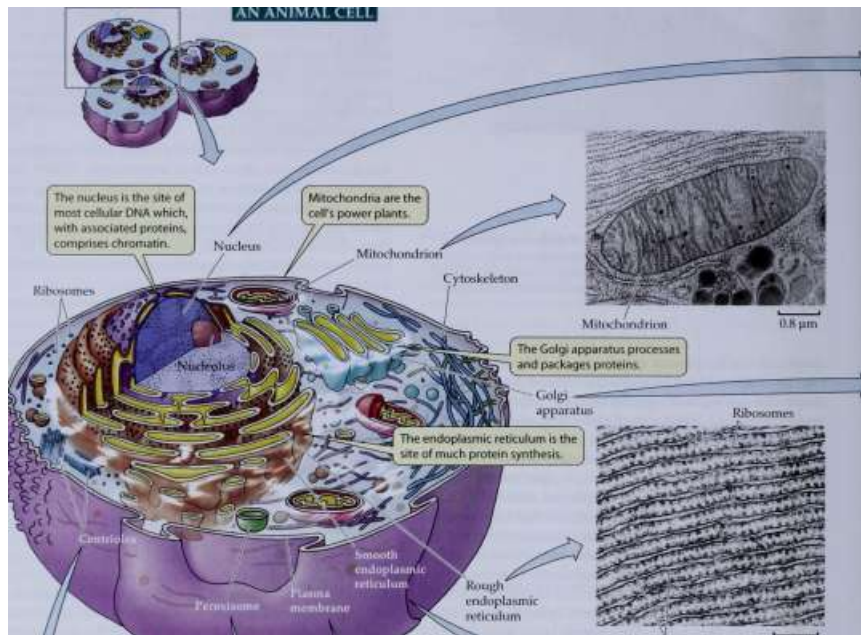
The flagellum is rotated by a complex protein 'motor' secured in the plasma membrane.

Flagellum

Hairlike pili help this bacterium adhere to other cells.

60 CHAPTER FOUR

AN ANIMAL CELL



Rough endoplasmic reticulum 0.5 urn

Centrioles are associated with nuclear division.

Peroxisomes break down toxic peroxides

irs



•ioles

0.1 urn



4.7 Eukaryotic Cells

n micrographs, many plant cell organelles are nearly identical those observed in animal cells. Cellular structures unique to plan :iude the cell wall and the chloroplasts. Animal cells contain

centrioles, which are not found in plant cells.

Outside of cell

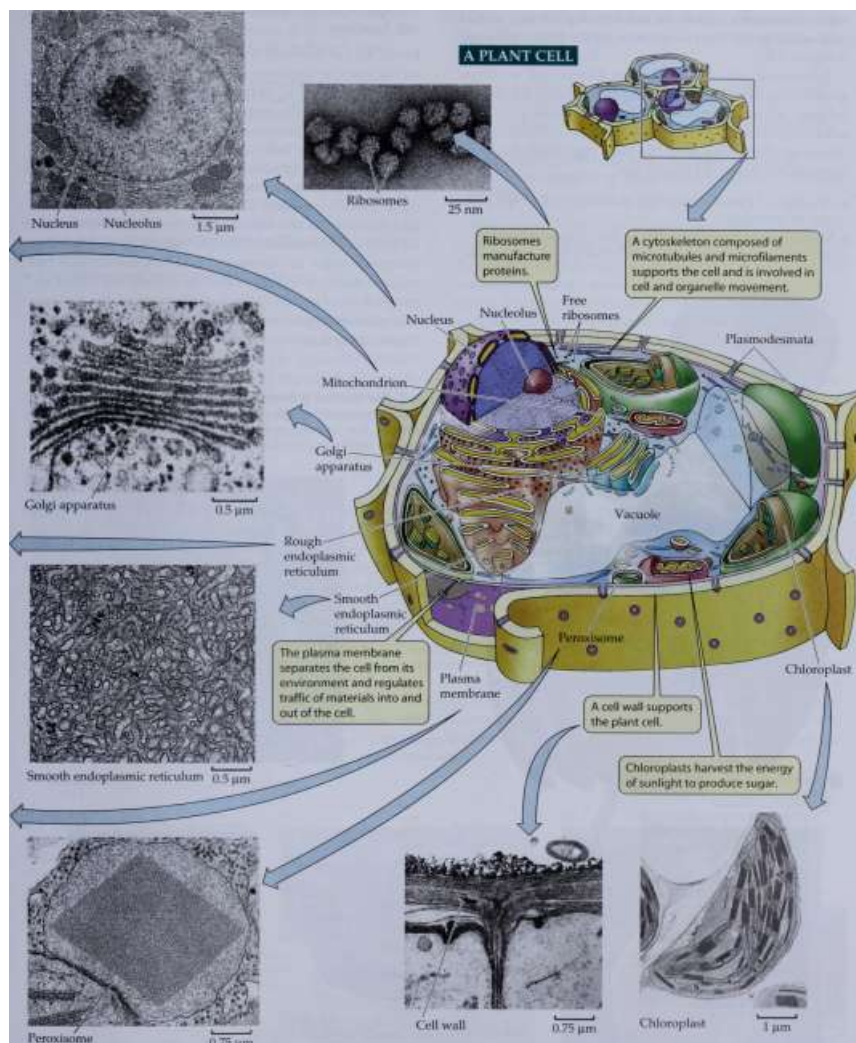


Plasma membrane

30 nm

^

THE ORGANIZATION OF CELLS 61



Peroxisome

0.75 |xm

62 CHAPTER FOUR

nent differences, compare the eukaryotic plant and animal cells on the preceding two pages with the prokaryotic cell in Figure 4.4.

Eukaryotic cells generally have dimensions ten times greater than those of prokaryotes; for example, the spherical yeast cell has a diameter of 8 urn. Like prokaryotic cells, eukaryotic cells have a plasma membrane, cytoplasm, and ribosomes. But added on to this basic organization are two elements not found in prokaryotes:

- An internal cytoskeleton that maintains cell shape and moves materials
- Membranous compartments in the cytoplasm whose interiors are separated from the cytosol by a membrane

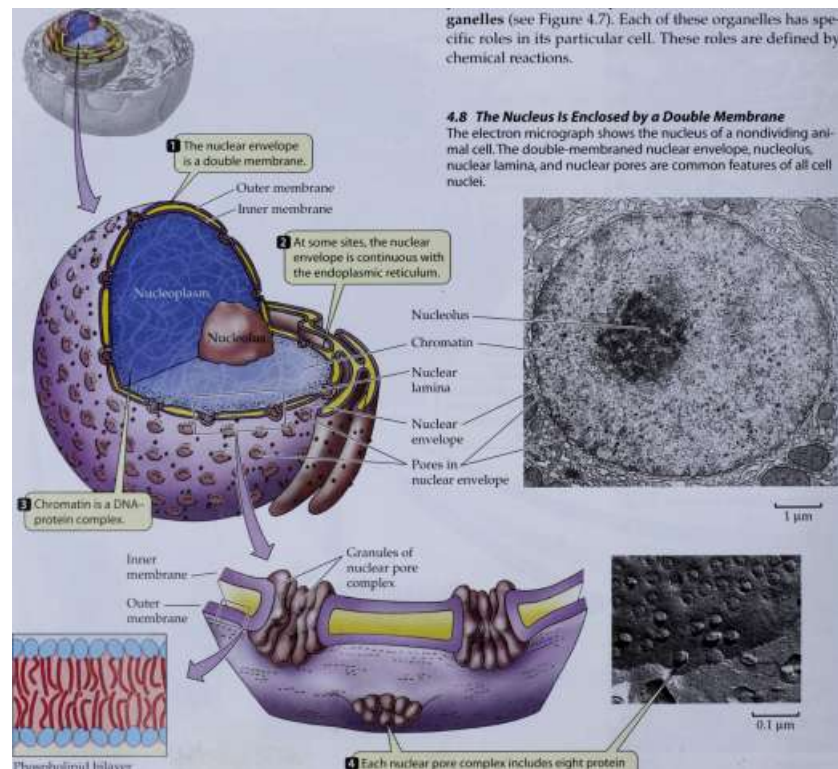
Compartmentalization is the key to eukaryotic cell function

Recall that prokaryotic cells are surrounded by a plasma membrane that regulates molecular traffic into and out of the cell. In addition, eukaryotic cells have "cells within cells"—interior compartments surrounded by membranes that regulate what enters or leaves that compartment. The membranes ensure that conditions inside the compartment are different from those in the surrounding cytoplasm.

Some of the compartments are like little factories that make specific products. Others are like power plants that take in energy in one form and convert it to a more useful form. These membranous compartments, as well as other structures (such as ribosomes) that lack membranes but possess distinctive shapes and functions, are called organelles (see Figure 4.7). Each of these organelles has specific roles in its particular cell. These roles are defined by chemical reactions.

4.8 The Nucleus Is Enclosed by a Double Membrane

The electron micrograph shows the nucleus of a nondividing animal cell. The double-membraned nuclear envelope, nucleolus, nuclear lamina, and nuclear pores are common features of all cell nuclei.



0.1 urn

Phospholipid bilayer

Each nuclear pore complex includes eight protein granules surrounding a pore, through which proteins from the cytoplasm enter the nucleus and RNA from the nucleus passes into the cytoplasm.

THE ORGANIZATION OF CELLS 63

- ▶ The nucleus contains most of the cell's genetic material (DNA). It determines the expression of this material as cell functions and its duplication when the cell reproduces.
- ▶ The mitochondrion is a power plant and industrial park, where energy stored in the bonds of carbohydrates is converted to a form more useful to the cell and certain essential biochemical conversions of amino acids and fatty acids occur.
- ▶ The endoplasmic reticulum and Golgi apparatus make up a compartment where proteins are packaged and sent to appropriate locations in the cell.
- ▶ The lysosome and vacuole are cellular digestive systems, where large molecules are hydrolyzed into usable monomers.
- ▶ The chloroplast performs photosynthesis.

All of these organelles have unique chemical compositions and functions. The membrane surrounding each does two essential things: First, it keeps the organelle's molecules away from other molecules in the cell with which they might react inappropriately. Second, it acts as a traffic regulator, letting important raw materials into the organelle and releasing its products to the cytoplasm.

Organelles that Process Information

Living things depend on accurate, appropriate information—internal signals, environmental cues, and stored instructions—to respond appropriately to changing conditions and maintain a constant internal environment. In the cell, information is stored as the sequence of nucleotides in DNA molecules. Most DNA in eukaryotic cells resides in the nucleus. Information is translated from the language of DNA into the language of proteins at the ribosomes. This process is described in detail in Chapter 12.

The nucleus stores most of the cell's DNA

The single nucleus is usually the largest organelle in a cell (Figure 4.8; see also Figure 4.7). The nucleus of most animal cells is approximately 5 µm in diameter—substantially larger than most entire prokaryotic cells. The nucleus has several roles in the cell:

- ▶ The nucleus is the site of DNA duplication to support cell reproduction.

► The nucleus is the site of DNA control of cellular activities.

► A region within the nucleus, called the nucleolus, begins the assembly of ribosomes from specific proteins and RNA.

The nucleus is surrounded by two membranes, which together form the nuclear envelope. The two membranes of the nuclear envelope are separated by only a few tens of nanometers and are perforated by nuclear pores approximately 9 nm in diameter, which connect the interior of the nucleus with the cytoplasm. At these pores, the outer membrane of the nuclear envelope is continuous with the inner membrane. Each pore is surrounded by a pore complex: eight large protein granules arranged in an octagon where the inner and outer membranes merge. RNA and proteins pass through these pores to enter or leave the nucleus.

At certain sites, the outer membrane of the nuclear envelope folds outward into the cytoplasm and is continuous with the membrane of another organelle, the endoplasmic reticulum (discussed later in the chapter).

Inside the nucleus, DNA combines with proteins to form a fibrous complex called chromatin. These are exceedingly long, thin, entangled threads that, prior to cell division, condense to form readily visible objects called chromosomes (Figure 4.9).

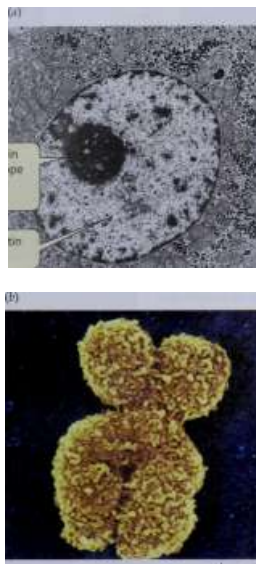
Surrounding the chromatin are water and dissolved substances collectively referred to as the nucleoplasm. Within the nucleoplasm, a network of apparently structural proteins called the nuclear matrix organizes the chromatin. At the periphery of the nucleus, the chromatin attaches to a protein meshwork, called the nuclear lamina, which is formed by the polymerization of proteins called lamins into filaments (Figure 4.10). The nuclear lamina maintains the

4.9 Chromatin and Chromosomes

(a) When a cell is not dividing, the nuclear DNA and proteins are aggregated as chromatin, which is dispersed throughout the nucleus, (b) The chromatin in a dividing cell is packed into dense bodies called chromosomes.

There is dense chromatin near the nuclear envelope attached to the nuclear lamina.

There is diffuse chromatin in the nucleoplasm.

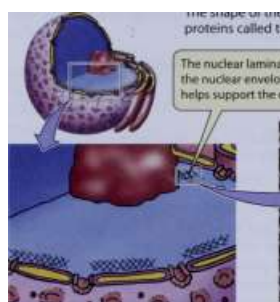


1 |im

0.5 |im

64 CHAPTER FOUR

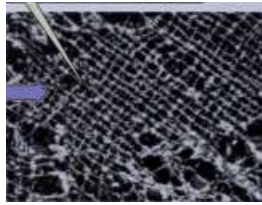
1



4.70 The Nuclear Lamina

The shape of the nucleus is maintained by a meshwork of proteins called the nuclear lamina.

The nuclear lamina is a network of filaments just inside the nuclear envelope. It interacts with chromatin and helps support the envelope to which it is attached.



lopes and the endomembrane system are visible by electron microscopy. Tiny vesicles appear to shuttle between the various components of the endomembrane system. This system has various structures, but all of them are essentially compartments, closed off by their membranes from the cytoplasm.

In this section, we will examine the functional significance of these compartments, and show that materials synthesized in the endoplasmic reticulum can be transferred to another organelle, the Golgi apparatus, for further processing, storage, or transport. We will also describe the role of the lysosome in cell digestion.

shape of the nucleus by its attachment to both chromatin and the nuclear envelope.

During most of the life cycle of the cell, the nuclear envelope is a stable structure. When the cell divides, however, the nuclear envelope fragments into pieces of membrane with attached pore complexes. The envelope re-forms when distribution of the duplicated DNA to the daughter cells is completed.

Ribosomes are the sites of protein synthesis

In both eukaryotic and prokaryotic cells, proteins are synthesized on thousands of ribosomes. Ribosomes are tiny granules found in three places in almost all eukaryotic cells: free in the cytoplasm, attached to the surface of endoplasmic reticulum (as will be described later in this chapter), and inside the mitochondria, where energy is processed. Ribosomes are also found in chloroplasts, the photosynthetic organelles of plant cells. In each of these locations, the ribosomes provide the sites where proteins are synthesized under the direction of nucleic acids. Although they seem small in comparison to the cell in which they are contained, ribosomes are huge machines composed of several dozen kinds of molecules.

The ribosomes of prokaryotes and of eukaryotes are similar in that both consist of two different-sized subunits. Eukaryotic ribosomes are somewhat larger, but the structure of prokaryotic ribosomes is better understood. Chemically, ribosomes consist of a special type of RNA, called ribosomal RNA, to which more than 50 different protein molecules are noncovalently bound.

The Endomembrane System

Much of the volume of some eukaryotic cells is taken up by an extensive endomembrane system. This system includes two main components, the endoplasmic reticulum and the Golgi apparatus. Continuities between the nuclear envelope

0.25 μm

The endoplasmic reticulum is a complex factory

Electron micrographs reveal a network of interconnected membranes branching throughout the cytoplasm, forming tubes and flattened sacs. These membranes are collectively called the endoplasmic reticulum, or ER. The interior compartment of the ER, referred to as the lumen, is separate and distinct from the surrounding cytoplasm (Figure 4.11). The surface area of the ER can occupy up to 15 percent of the entire interior volume of the cell, and its foldings result in a surface area many times greater than that of the plasma membrane. At certain sites, the ER is continuous with the outer membrane of the nuclear envelope.

Parts of the ER are liberally sprinkled with ribosomes, which are temporarily attached to the outer faces of the flattened sacs. Because of their appearance in the electron microscope, these regions are called rough ER, or RER. RER has two roles:

- As a compartment, it segregates certain newly synthesized proteins away from the cytoplasm and transports them to other locations in the cell.
- While inside the RER, proteins can be chemically modified so as to alter their function and intracellular destination.

The attached ribosomes are sites for the synthesis of proteins that function outside the cytosol—that is, proteins that are to be exported from the cell, incorporated into membranes, or moved into organelles of the endomembrane system. These proteins enter the lumen of the ER as they are synthesized. Once in the lumen of the ER, these proteins undergo several changes, including the formation of disulfide bridges and folding into their tertiary structures (see Figure 3.5). Proteins gain carbohydrate groups in the RER, thus becoming glycoproteins. The carbohydrate groups are part of an "addressing" system that ensures that the right proteins are directed to the right parts of the cell.

Some parts of the endoplasmic reticulum, called the smooth ER or SER, are more tubular (less like flattened

THE ORGANIZATION OF CELLS 65

sacs) and lack ribosomes (see Figure 4.11). Within the lumen of the SER, proteins that have been synthesized on the RER are

chemically modified. In addition, the SER has two other important roles:

- It is responsible for chemically modifying small molecules taken in by the cell. This is especially true for drugs and pesticides.
- It is the site for the hydrolysis of glycogen and the synthesis of steroids.

Cells that synthesize a lot of protein for export are usually packed with ER. Examples include glandular cells that secrete digestive enzymes and plasma cells that secrete antibodies. In contrast, cells that carry out less protein synthesis (such as storage cells) contain less ER.

The Golgi apparatus stores, modifies, and packages proteins

In 1898, the Italian microscopist Camillo Golgi discovered a delicate structure in nerve cells, which came to be known as the Golgi apparatus. Because of the resolution limits of light microscopy, and because the staining techniques of the time often failed to reveal the structure, many biologists regarded it as a product of Golgi's imagination. In the late 1950s, however, the electron microscope showed clearly that the Golgi apparatus does exist—and not just in nerve cells, but in most eukaryotic cells.

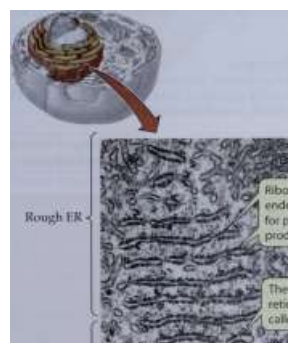
The exact appearance of the Golgi apparatus varies from species to species, but it always consists of flattened membranous sacs called cisternae and small membrane-enclosed vesicles. The cisternae appear to be lying together like a stack of saucers (Figure 4.12a). The entire apparatus is about 1 μm long.

The Golgi apparatus has several roles:

- It receives proteins from the ER and chemically modifies them.
- Proteins within the Golgi apparatus are concentrated, packaged, and sorted before being sent to their cellular or extracellular destinations.
- The Golgi apparatus is where some polysaccharides for the plant cell wall are synthesized.

In the cells of plants, protists, fungi, and many invertebrate animals, the stacks of cisternae are individual units scattered throughout the cytoplasm. In vertebrate cells, a few such stacks usually form a larger, single, more complex Golgi apparatus.

The Golgi appears to have three functionally distinct parts: a bottom, a middle, and a top. The bottom cisternae, constituting the cis region of the Golgi apparatus, lie nearest to the nucleus or a patch of RER (see Figure 4.12). The top cisternae, constituting the trans region, lie closest to the surface of the cell. The cisternae in the middle make up the medial region of the complex. These three parts of the Golgi apparatus contain different enzymes and perform different functions.



4.11 Endoplasmic Reticulum

The transmission electron micrograph on the left shows a two-dimensional slice through the three-dimensional structures depicted in the drawing. In normal living cells, membranes never have open ends; they define closed compartments set off from the surrounding cytoplasm.

Smooth ER *

.-,"j/

\w



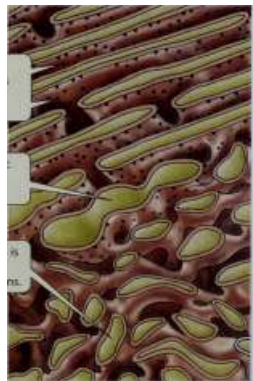
Ribosomes of the rough endoplasmic reticulum are sites for protein synthesis. They produce its rough appearance. The interior of the endoplasmic reticulum compartment is called the lumen.

Smooth endoplasmic reticulum is a site for lipid synthesis and chemical modification of proteins

UjtfH

>%** **^*#

0.5 urn



> Rough ER

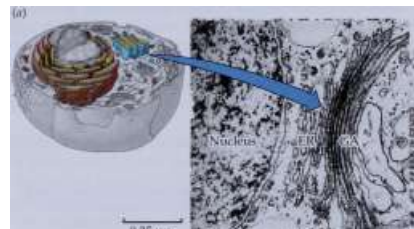
> Smooth ER

66 CHAPTER FOUR

The Golgi apparatus receives proteins from the ER, packages them, and sends them on their way. The chemical modifications made to proteins within the Golgi apparatus generally "tag" them to their proper destinations—a process we will describe further in Chapter 12. So in some sense the Golgi apparatus is a "post office" for the cell.

Since there is often no direct membrane continuity between ER and Golgi apparatus, how does a protein get from one organelle to the other? The protein could simply leave the ER, travel across the cytoplasm, and enter the Golgi apparatus. But this would expose the protein to interactions with other molecules in the cytoplasm. On the other hand, segregation from the cytoplasm could be maintained if a piece of the ER could "bud off," forming a vesicle that contains the target protein—and this is in fact exactly what happens. The protein makes the passage from ER to Golgi apparatus safely enclosed in the vesicle. Once it arrives, the vesicle fuses with the membrane of the Golgi apparatus, releasing its cargo.

Vesicles form from the rough ER, move through the cytoplasm, and fuse with the cis region of the Golgi appara-



0.25 urn *&•*

-**-' ;, wfc

(b)

I Protein-containing vesicles from the endoplasmic reticulum transfer substances to the Golgi apparatus.

| The endoplasmic reticulum vesicles fuse with the cis region of the Golgi.

tus, where their contents are released into the lumen of the Golgi. Other small vesicles may move between the cisternae, transporting proteins. Associated with the cisternae, particularly those toward the trans region, are tiny vesicles that pinch off and move to other cisternae or away from the Golgi (see Figure 4.12b).

The membranes of two vesicles can sometimes make contact with each other and fuse, resulting in a larger vesicle and a mixing of the contents. Vesicles may also fuse with other organelles, or with the plasma membrane, where they release their contents to the outside of the cell. The formation, transport, and fusing behavior of vesicles is essential to the function of the Golgi apparatus. Structurally, vesicles are the transport vehicles into and out of the Golgi apparatus and to the ultimate destinations of the proteins.

Lysosomes contain digestive enzymes

Originating in part from the Golgi apparatus are organelles called lysosomes. They contain digestive enzymes, and they are the sites of hydrolysis of macromolecules—proteins, polysaccharides, nucleic acids, and lipids—to their monomers (see Figure

3.2). Lysosomes are about 1 urn in diameter, are surrounded by a single membrane, and have a densely staining, featureless interior (Figure 4.13fl). There may be dozens of lysosomes in a cell, depending on its needs.

Lysosomes are sites for the breakdown of food and foreign objects taken up by the cell. How do these materials get into the cell in the first place? In a process called phagocytosis (phago-, "eating"; cytosis, "cellular"), a pocket forms in the plasma membrane and eventually deepens and en-

4.12 The Golgi Apparatus

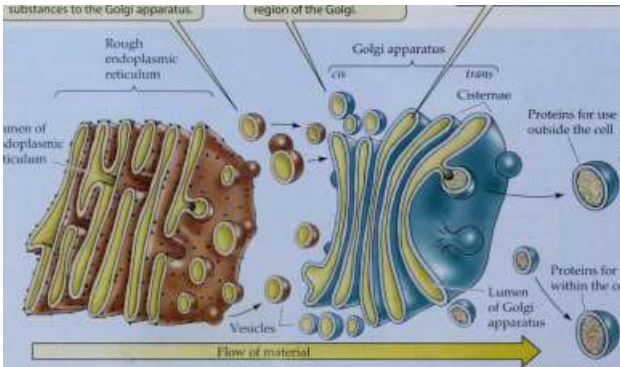
(a) The Golgi apparatus appears as stacked disks in an electron micrograph, (b) The Golgi apparatus modifies proteins from the ER and "targets" them to the correct addresses.

f

The Golgi chemically modifies proteins in its lumen...

. .and "targets" them to the correct addresses.

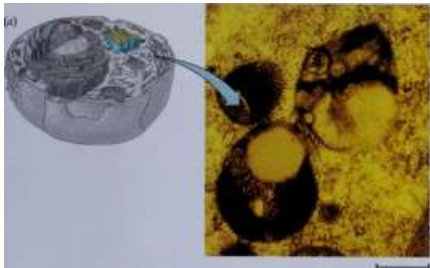
Lumen of endoplasmic reticulum .^J



Proteins for use within the cell



THE ORGANIZATION OF CELLS 67



rn



4.13 Lysosomes Isolate Digestive Enzymes from the Cytoplasm

(a) In this electron micrograph of a rat cell, the darkly stained organelles are secondary lysosomes in which digestion is taking place, (b) The origin and action of lysosomes and lysosomal digestion.

(b)

0.5 urn

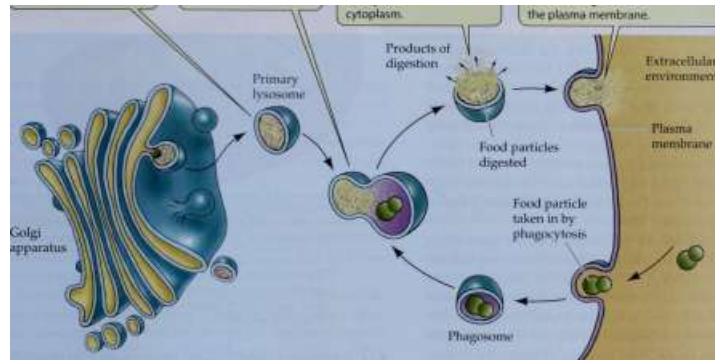
t

The primary lysosome is generated by the Golgi.

Q The lysosome fuses with I a phagosome

§J Small molecules generated by digestion diffuse into the cytoplasm.

o Undigested materials are released when the digestion vesicle fuses with the plasma membrane.



closes material from outside the cell. This pocket becomes a small vesicle and breaks free of the plasma membrane to move into the cytoplasm as a phagosome containing food or other material (Figure 4.13b). The phagosome fuses with a primary lysosome to form a secondary lysosome, where digestion occurs.

The effect of this fusion is rather like releasing hungry foxes into a chicken coop. The enzymes in the secondary lysosome quickly hydrolyze the food particles. These reactions are enhanced by the mild acidity of the lysosome's interior, where the pH is lower than in the surrounding cytoplasm. The products of digestion exit through the membrane of the lysosome, providing fuel molecules and raw materials for other cell processes. The "used" secondary lysosome containing undigested particles then moves to the plasma membrane, fuses with it, and releases the undigested contents to the environment.

Lysosomes are also where the cell digests its own material in a process called autophagy. Autophagy is an ongoing process, in which macromolecules such as proteins are en-

gulfed by lysosomes and hydrolyzed to amino acids, which pass out of the lysosome through its membrane into the cytoplasm for reuse.

Plant cells do not appear to contain lysosomes, but the central vacuole of a plant cell may function in an equivalent capacity because it, like lysosomes, contains many digestive enzymes.

Organelles that Process Energy

A cell uses energy to transform raw materials into cell-specific materials that it can use for activities such as growth, reproduction, and movement. Energy is transformed from one form to another in mitochondria (found in all eukaryotic cells) and in chloroplasts (found in eukaryotic cells that harvest energy from sunlight). In contrast, energy transformations in prokaryotic cells are associated with enzymes attached to the inner surface of the plasma membrane or extensions of the plasma membrane that protrude into the cytoplasm.

68 CHAPTER FOUR

Mitochondria are energy transformers

In eukaryotic cells, the utilization of food molecules such as glucose begins in the cytosol. The fuel molecules that result from partial degradation of this food enter the mitochondria (singular mitochondrion), whose primary function is to convert the potential chemical energy of fuel molecules into a form that the cell can use: the energy-rich molecule called ATP, or adenosine triphosphate. ATP is not a long-term energy storage form, but rather a kind of energy currency. Its role in the cell is analogous to the role of paper money in an economy. Chemically, ATP can participate in a great number of different cellular reactions and processes that require energy. In the mitochondria, the production of ATP using fuel molecules and O_2 is called cellular respiration.

Typical mitochondria are small—somewhat less than 1.5 urn in diameter and 2-8 urn in length—about the size of many bacteria. Mitochondria are visible with a light microscope, but almost nothing was known of their precise structure until they were examined with the electron microscope. Electron micrographs revealed that mitochondria have two membranes. The outer membrane is smooth and protective, and it offers little resistance to the movement of substances into and out of the mitochondrion. Immediately inside the outer mitochondrial membrane is an inner membrane, which folds inward in many places, giving it a much greater surface area than that of the outer membrane (Figure 4.14). These folds tend to be quite regular, giving rise to shelflike structures called cristae.

The inner mitochondrial membrane contains many large protein molecules that participate in cellular respiration and the production of ATP. The inner membrane exerts much more control over what enters and leaves the mitochondrion than does

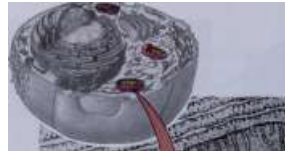
the outer membrane. The region enclosed by the inner membrane is referred to as the mitochondrial matrix. In addition to many proteins, the matrix contains some ribosomes and DNA that are used to make some of the proteins needed for cellular respiration.

The number of mitochondria per cell ranges from one contorted giant in some unicellular protists to a few hundred thousand in large egg cells. An average human liver cell contains more than a thousand mitochondria. Cells that require the most chemical energy tend to have the most mitochondria per unit of volume. In Chapter 7 we will see how the different parts of the mitochondrion work together in cellular respiration.

Plastids photosynthesize or store materials

One class of organelles—the plastids—is produced only in plants and certain protists. There are several types of plastids, with different functions.

chloroplasts. The most familiar of the plastids is the chloroplast, which contains the green pigment chlorophyll and is the site of photosynthesis (Figure 4.15). In photosynthesis, light energy is converted into the chemical energy of bonds between atoms. The molecules formed in photosynthesis provide food for plants themselves and for other



0.6 um

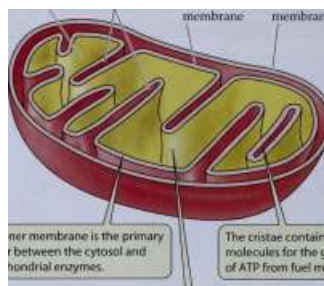
\$i

■ #

I v I \

Matrix Cristae Inner Outer

membrane membrane



The inner membrane is the primary barrier between the cytosol and mitochondrial enzymes.

The cristae contain key molecules for the generation of ATP from fuel molecules.

The matrix—the space enclosed by the inner membrane—contains several of the enzymes used for cellular respiration. It also contains ribosomes and DNA.

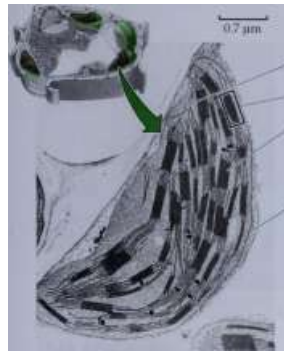
4.14 A Mitochondrion Converts Energy from Fuel Molecules into ATP

The electron micrograph is a two-dimensional slice through a three-dimensional reality. As the drawing emphasizes, the cristae are extensions of the inner mitochondrial membrane.

organisms that eat plants. Directly or indirectly, photosynthesis is the energy source for most of the living world.

Chloroplasts are quite variable in size and shape (Figure 4.16a, fr). Like the mitochondrion, the chloroplast is surrounded by two membranes. Arising from the inner membrane is a series of discrete internal membranes whose structure and arrangement vary from one group of photo-synthetic organisms to another. Here we concentrate on the chloroplasts of the flowering plants. Even these show some variation, but the pattern shown in Figure 4.15 is typical.

As seen in electron micrographs, the internal membranes of chloroplasts look like stacks of flat, hollow pita bread. These stacks, called grana (singular granum), consist of a series of flat, closely packed, circular compartments called thylakoids. In addition to phospholipids and proteins, the membranes of the thylakoids contain molecules



Stroma

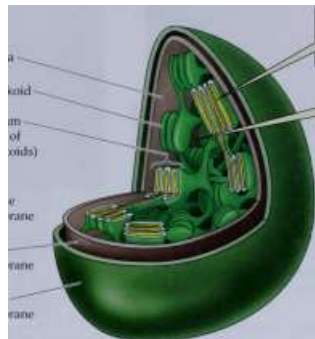
Thylakoid

Granum (stack of thylakoids)

Double membrane

Inner membrane

Outer membrane



Thylakoid membranes are sites where light energy is harvested by the green pigment chlorophyll and converted into ATP.

ATP converts CO_2 to glucose in the stroma, the area outside the thylakoid membranes.

4.75 The Chloroplast: The Organelle That Feeds the World

The electron micrograph shows a chloroplast from a leaf of corn. Chloroplasts are large compared with mitochondria and contain an extensive network of photo-synthetic thylakoids surrounded by two membranes.

of the green pigment chlorophyll and the yellow-orange carotenoids. These two pigment families harvest light for photosynthesis. Thylakoids of one granum may be connected to those of other grana, making the interior of the chloroplast a highly developed network of membranes, much like the ER.

The fluid in which the grana are suspended is referred to as stroma. Like the mitochondrial matrix, the chloroplast stroma contains ribosomes and DNA, and these are used to synthesize some, but not all, of the proteins that make up the chloroplast.

Animal cells do not produce chloroplasts, but some do contain functional chloroplasts. These are either taken up as free chloroplasts derived from the partial digestion of green plants, or contained within unicellular algae that live within the animal's tissues. The green color of some corals and sea anemones results from chloroplasts in algae that live within those animals (Figure 4.16c). The animals derive some of

Chloroplasts

Leaf cell

The chloroplasts in this single-celled green alga have assembled into a spiral.

their nutrition from the photosynthesis that their chloro-plast-containing "guests" carry out. Such an intimate relationship between two different organisms is called symbiosis.

other types of plastids. The red color of a flower or a ripe tomato results from the presence of legions of plastids called chromoplasts (Figure 4.17a). Just as chloroplasts derive their color from the pigment chlorophyll, chromoplasts are red, orange, or yellow depending on the kinds of carotenoid pigments present. The chromoplasts have no known chemical function in the cell, but the colors they give to some petals and fruits probably help attract animals that assist in pollination or seed dispersal. (On the other hand, carrot roots gain no apparent advantage from being orange.)

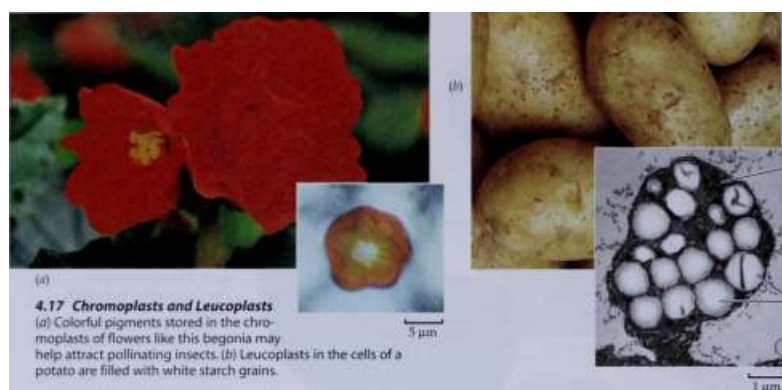
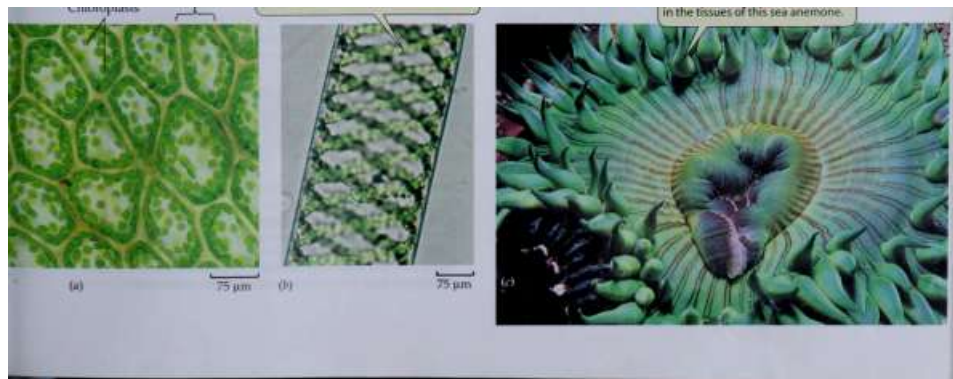
4.76 Being Green

(a) In green plants, chloroplasts are concentrated in the leaf cells.

(b) Green algae are photosynthetic and filled with chloroplasts.

(c) No animal species produces its own chloroplasts, but in this symbiotic arrangement, unicellular green algae nourish a giant sea anemone.

Chloroplast-filled green algae live in the tissues of this sea anemone.



Leucoplast

4.17 Chromoplasts and Leucoplasts

(a) Colorful pigments stored in the chromoplasts of flowers like this begonia may help attract pollinating insects. (b) Leucoplasts in the cells of a potato are filled with white starch grains.

Starch grains

Other plastids, called leucoplasts, are storage depots for starch and fats (Figure 4.17b).

Mitochondria and chloroplasts may have an endosymbiotic origin

Chloroplasts and mitochondria are about the size of prokaryotic cells. They contain DNA and have ribosomes that are similar to prokaryotic ribosomes, and they reproduce and divide within the cell to produce additional mitochondria and chloroplasts.

But, these organelles, even though they have the genetic material and protein synthesis machinery needed to make some of their own components, are not independent of control by the nucleus. The vast majority of their proteins are encoded by nuclear DNA, made in the cytoplasm, and imported into the organelle. These observations have led to speculation on the origin of these organelles. One proposal for this origin is the endosymbiosis theory of the origin of mitochondria and chloroplasts, which envisions the following scenario.

About 2 billion years ago, only prokaryotes inhabited Earth. Some of them absorbed their food directly from the environment. Others were photosynthetic. Still others fed on smaller prokaryotes by engulfing them (Figure 4.18).

Suppose that a small, photosynthetic prokaryote was ingested by a larger one, but was not digested. Instead, it survived trapped within a vesicle in the cytoplasm of the larger cell. The smaller, ingested prokaryote divided at about the same rate as the larger one, so successive generations of the larger cell also contained the offspring of the smaller one. We call this phenomenon endosymbiosis (endo-, "within"; symbiosis, "living together"); it is comparable to the algae that live within sea anemones (see Figure 4.16c).

According to this scenario, endosymbiosis provided benefits for both organisms. The larger cell obtained the photosynthetic products from the smaller cell, and the smaller cell was protected by the larger one. The smaller cell gradually lost much of its DNA to the nucleus, resulting in the modern chloroplast.

Much circumstantial evidence favors the endosymbiosis theory. Chloroplast DNA sequences are more like certain prokaryotic sequences than like any plant DNA. Moreover, on an evolutionary time scale of millions of years, there is

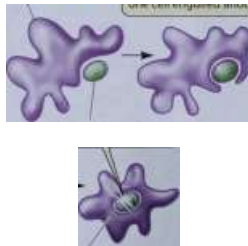
evidence for DNA moving between organelles in a cell. Finally, there are many biochemical similarities between chloroplasts and modern bacteria.

Similar evidence and arguments also support the proposition that mitochondria are the descendants of respiring prokaryotes engulfed by larger prokaryotes. The benefits of this endosymbiotic relationship might have been due to the capacity of the engulfed prokaryote to detoxify molecular oxygen (O_2), which was increasing in Earth's atmosphere because of photosynthesis.

However, mitochondria and chloroplasts are not enough to turn a prokaryote into a eukaryote. The endosymbiosis theory is still incomplete. For example, the origins of the nuclear envelope and other important structures—including those responsible for nuclear division—still need to be understood. We discuss further aspects of the origin of the eukaryotic cell in Chapter 27.

Membrane of larger cell

Double membranes may have originated when one cell engulfed another.

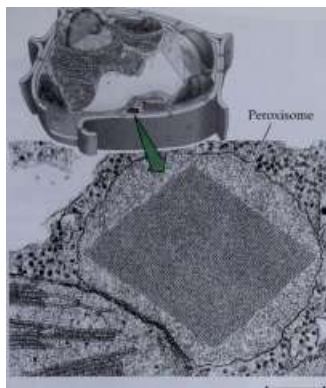


Membrane of smaller cell

Double membrane

4.18 The Endosymbiosis Theory

The double membrane that encloses mitochondria and chloroplasts may have arisen from two different sources: the outer membrane from the engulfing cell's plasma membrane and the inner membrane from the engulfed cell's plasma membrane.



A diamond-shaped crystal, composed of an enzyme, almost entirely fills this rounded peroxisome in a leaf cell. The enzyme catalyzes one of the reactions fulfilling the special function of the peroxisome.

Other Organelles

In addition to the information-processing organelles (nucleus and ribosomes), the energy-processing organelles (mitochondria and chloroplasts), and the organelles of the endomembrane system (endoplasmic reticulum, Golgi apparatus, and lysosomes), there are two other kinds of membrane-enclosed organelles: peroxisomes and vacuoles. Both are surrounded by a single membrane.

Peroxisomes house specialized chemical reactions

Peroxisomes are small organelles—0.2 to 1.7 μm in diameter. They have a single membrane and a granular interior (Figure 4.19). Peroxisomes are found at one time or another in at least some of the cells of almost every eukaryotic species. Peroxisomes are organelles within which toxic peroxides (such as hydrogen peroxide, H_2O_2) are formed as unavoidable side products of chemical reactions. Subsequently, the peroxides are safely broken down within the peroxisomes without mixing with other parts of the cell.

A structurally similar organelle, the glyoxysome, is found only in plants. Glyoxysomes, which are most prominent in young plants, are the sites where stored lipids are converted into carbohydrates for transport to growing cells.

Vacuoles are filled with water and soluble substances

Many eukaryotic cells, but particularly those of plants and protists, contain membrane-enclosed organelles that look empty under the electron microscope. These organelles are called vacuoles (Figure 4.20). They are not actually empty; rather, they are filled with aqueous solutions that contain many dissolved substances.

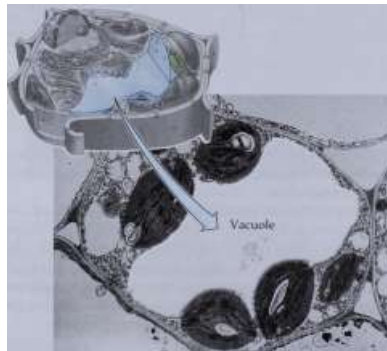
THE ORGANIZATION OF CELLS 71

Despite their structural simplicity, vacuoles have a variety of functions. For example, like animals and other organisms, plant cells produce a number of toxic by-products and waste materials. Animals have specialized excretory mechanisms for getting rid of such wastes, but plants do not. Although plants can secrete some wastes to their environment, many are simply stored within vacuoles. And since they are poisonous or distasteful, these stored materials deter some animals from eating the plants. Thus stored wastes may contribute to plant survival.

In many plant cells, enormous vacuoles take up more than 90 percent of the cell volume and grow as the cell grows. But vacuoles are by no means a waste of space, for the dissolved substances in the vacuole, working together with the vacuolar membrane, provide the turgor, or stiffness, of the cell, which in turn provides support for the structure of nonwoody plants. The presence of the dissolved substances causes water to enter the vacuole, making it tend to swell like a balloon. Plant cells have a rigid cell wall, which acts like a box, resisting the swelling of the vacuole but providing strength in the process.

Vacuoles even play a role in the sex life of plants. Some pigments (especially blue and pink ones) in petals and fruits are contained in vacuoles. These pigments—the anthocyanins—are visual cues that encourage animals to visit flowers and thus aid in pollination, or to eat fruits and thus aid in seed dispersal.

Food vacuoles are found in some simple and evolutionarily ancient groups of organisms: single-celled protists and simple multicellular organisms such as sponges. In these organisms, the cells engulf food particles by phagocytosis, generating a food vacuole. Fusion of this vacuole with a



2|im

4.20 Vacuoles in Plant Cells Are Usually Large

The large central vacuole in this cell is typical of mature plant cells. Smaller vacuoles are visible toward each end of the cell.

72 CHAPTER FOUR

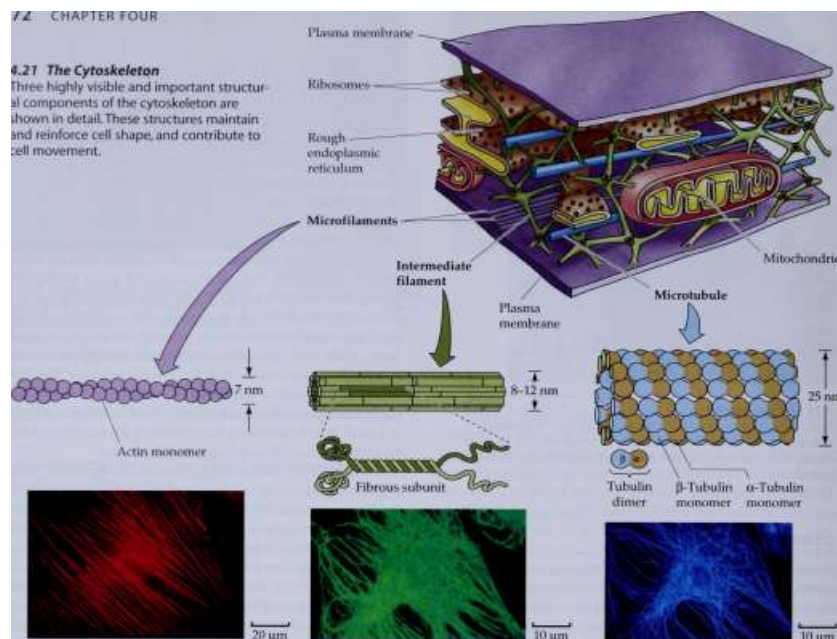
4.27 The Cytoskeleton

Three highly visible and important structural components of the cytoskeleton are shown in detail. These structures maintain and reinforce cell shape, and contribute to cell movement.

Plasma membrane

Ribosomes

Mitochondrion



25 nm

Microfilaments are made up of strands of the protein actin and often interact with strands of other proteins. They change cell shape and drive cellular motion, including contraction, cytoplasmic streaming, and the "pinched" shape changes that occur during cell division. Microfilaments and myosin strands together drive muscle action.

Intermediate filaments are made up of fibrous proteins organized into tough, ropelike assemblages that stabilize a cell's structure and help maintain its shape. Some intermediate filaments hold neighboring cells together. Others make up the nuclear lamina.

Microtubules are long, hollow cylinders made up of many molecules of the protein tubulin. Tubulin consists of two subunits, α -tubulin and β -tubulin. Microtubules lengthen or shorten by adding or subtracting tubulin dimers. Microtubule shortening moves chromosomes. Interactions between microtubules drive the movement of cells. Microtubules serve as "tracks" for the movement of vesicles.

lysosome results in digestion, and small molecules leave the vacuole and enter the cytoplasm for use or distribution to other organelles.

Many freshwater protists have a highly specialized contractile vacuole. Its function is to rid the cell of the excess water that rushes in because of the imbalance in salt concentration between the relatively salty interior of the cell and its freshwater environment. The contractile vacuole enlarges as water enters, then abruptly contracts, forcing the water out of the cell through a special pore structure.

The Cytoskeleton

In addition to the many membrane-enclosed organelles, the eukaryotic cytoplasm has a set of long, thin fibers called the cytoskeleton, which fills at least three important roles:

- It maintains cell shape and support.
- It provides for various types of cell movement.
- Some of its fibers act as tracks or supports for "motor proteins," which help the cell move or move things within the cell.

In the discussion that follows, we'll look at three components of the cytoskeleton: microfilaments, intermediate filaments, and microtubules (Figure 4.21).

Microfilaments function in support and movement

Microfilaments can exist as single filaments, in bundles, or in networks. They are about 7 nm in diameter and several μ m long. They are assembled from actin, a protein that ex-

ists in several forms and has many functions among members of the animal phyla. The actin found in microfilaments (which are also known as actin filaments) is extensively folded and has distinct "head" and "tail" sites. These sites interact with similar actin molecules to assemble into a long chain (see Figure 4.21). Two of these chains interact to form the double helix of a microfilament. The polymerization of actin into microfilaments is reversible, and they can disappear from cells, breaking down into units of free actin.

Microfilaments have two major roles:

- They help the entire cell or parts of the cell to contract.
- They stabilize cell shape.

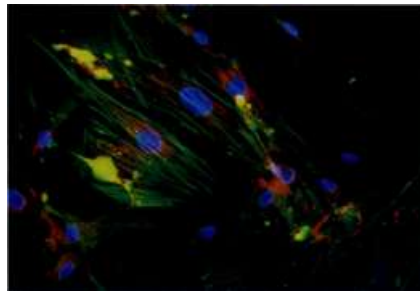
In muscle cells, actin fibers are associated with another protein called myosin, and their interactions account for the contraction of muscles. In nonmuscle cells, actin fibers are associated with localized changes of shape in cells. For example, microfilaments are involved in a flowing movement of the cytoplasm called cytoplasmic streaming, in movements of specific organelles and particles within cells, and in the "pinching" contractions that divide an animal cell into two daughter cells. Microfilaments are also involved in the formation of cellular extensions, called pseudopodia (pseudo-, "false;" podia, "feet"), that enable cells to move (Figure 4.22).

In some cell types, microfilaments form a meshwork just inside the plasma membrane. Actin-binding proteins then cross-link the microtubules to form a rigid structure that supports the



The microvilli of the cells lining the intestine enlarge the surface area over which nutrients can be absorbed.

o
c
o
o
'_ ^



4.22 Microfilaments for Motion 20 ^ m

The green-stained microfilaments in these cells provide a way for the cell to move.

A cap of proteins is attached to the end of microfilaments.

Actin microfilaments run the entire --1 length and support each microvillus.

Cross-linking actin-binding proteins link microfilaments to each other and to the plasma membrane.

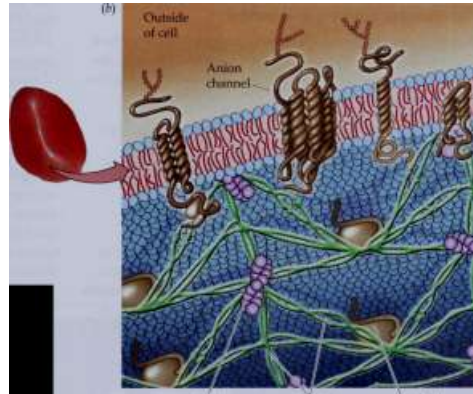
Plasma membrane



Intermediate filaments



0.25 μm



Actin

Spectrin

Ankyrin

4.23 Microfilaments for Support

(a) Microfilaments form the backbone of the microvilli that increase the surface area of some cells, such as intestinal cells that absorb nutrients, (b) Actin microfilaments, along with ankyrin and spectrin proteins, support the "doughnut" shape of red blood cells.

cell. For example, microfilaments support the tiny microvilli that line the intestine, giving it a larger surface area through which to absorb nutrients. Such a "submembrane skeleton" also helps keep the red blood cell in its familiar doughnut shape (Figure 4.23).

74 CHAPTER FOUR

Intermediate filaments are tough supporting elements

Intermediate filaments (see Figure 4.21) are found only in multicellular organisms. Although there are at least five distinct types of intermediate filaments, all share the same general structure and are composed of fibrous proteins of the keratin family, similar to the protein that makes up hair and fingernails. In cells, these proteins are organized into tough, ropelike assemblages 8 to 12 nm in diameter.

Intermediate filaments have two major structural functions:

- They stabilize cell structure.
- They resist tension.

In some cells, intermediate filaments radiate from the nuclear envelope and may maintain the positions of the nucleus and other organelles in the cell. The lamins of the nuclear lamina are intermediate filaments. Other kinds of intermediate filaments help hold a complex apparatus of microfilaments in place in muscle cells. Still other kinds stabilize and help maintain rigidity in surface tissues by connecting "spot welds" called desmosomes between adjacent cells (see Figure 5.6b).

Microtubules are long and hollow

Microtubules are long, hollow, unbranched cylinders about 25 nm in diameter and up to several micrometers long. Assembled from molecules of the protein tubulin, microtubules have two roles:

- They form a rigid internal skeleton for some cells, especially at cell extensions.

► They act as a framework on which motor proteins can move structures in the cell.

Tubulin is a dimer made up of two polypeptide monomers, called α -tubulin and β -tubulin. Thirteen rows, of tubulin dimers surround the central cavity of the microtubule (see Figure 4.21). The two ends of a microtubule are different. One end is designated the + end, the other the - end. Tubulin dimers can be added or subtracted mainly at the + end, lengthening or shortening the microtubule. This capacity to change length rapidly makes microtubules dynamic structures.

This dynamic property is seen in animal cells, where microtubules are often found in parts of the cell that are changing shape. Many microtubules radiate from a region of the cell called the microtubule organizing center. Tubule polymerization results in a rigid cell, and tubule depolymerization leads to a collapse of this rigid structure. In plants, microtubules help control the arrangement of the cellulose fibers of the cell wall. Electron micrographs of plants frequently show microtubules lying just inside the plasma membrane of cells that are forming or extending their cell walls. Experimental alteration of the orientation of these microtubules leads to a similar change in the cell wall, and a new shape for the cell. In many cells, microtubules serve as tracks for motor proteins, specialized molecules that use energy to change their shape and move. Motor proteins bond to and move along the microtubules, carrying materials from one part of the cell to another. Microtubules are also essential in distributing chromosomes to daughter cells during cell division. And they are intimately associated with movable cell appendages: the flagella and cilia.

Microtubules power cilia and flagella

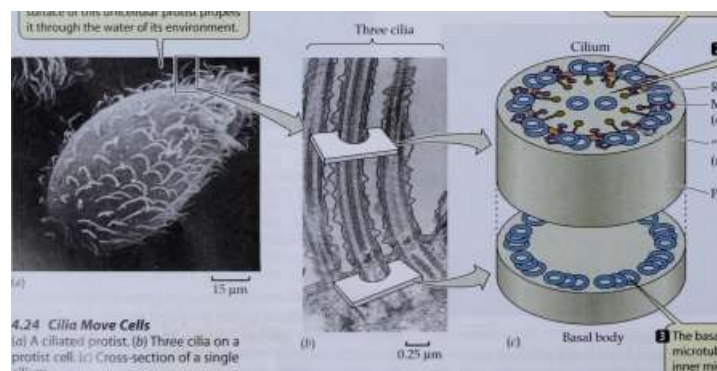
Many eukaryotic cells possess flagella and/or cilia. These whiplike organelles push or pull the cell through its aque-

The beating of the cilia covering the surface of this unicellular protist propels it through the water of its environment.

I Cross-section reveals the "9+2" pattern of microtubules, including nine pairs of fused microtubules...

Three cilia

A



4.24 Cilia Move Cells

{a) A ciliated protist. (b) Three cilia on a protist cell.(c) Cross-section of a single cilium.

...and two unfused inner microtubules.

Radial spoke Motor protein (dynein)

"Linker" (nexin)

protein

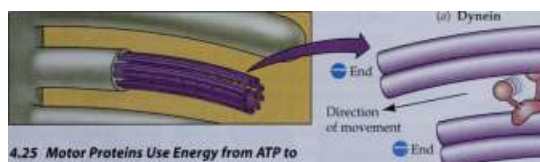
Plasma membrane

0.25 nm

| The basal body has nine fused microtubule triplets but no inner microtubules.

THE ORGANIZATION OF CELLS 75

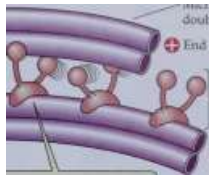
(a) Dynein



4.25 Motor Proteins Use Energy from ATP to Move Things

{a) Dynein operates in muscle contraction and flagellar movement, (b) Kinesin delivers vesicles to various parts of the cell. All

motor proteins work by undergoing reversible shape changes powered by energy from ATP. (c) The SEM shows a vesicle attached to a microtubule in a motor protein.



Microtubule doublet

Dynein

©End

Dynein is permanently attached to one microtubule and moves it with respect to a neighboring one.

ous environment, or they may move surrounding liquid over the surface of the cell (Figure 4.24a). Cilia and eukaryotic (but not prokaryotic*) flagella are both assembled from specialized microtubules and have identical internal structures, but they differ in their relative lengths and their patterns of beating:

► Flagella are longer than cilia and are usually found singly or in pairs. Waves of bending propagate from one end of a flagellum to the other in snakelike undulation.

► Cilia are shorter than flagella and are usually present in great numbers. They beat stiffly in one direction and recover flexibly in the other direction (like a swimmer's arm), so that the recovery stroke does not undo the work of the power stroke.

Observed by electron microscopy in cross section, a typical cilium or eukaryotic flagellum is surrounded by the plasma membrane and contains a "9 + 2" array of microtubules. As Figure 4.24b shows, nine fused pairs of microtubules—called doublets—form an outer cylinder, and one pair of unfused microtubules runs up the center. A spoke radiates from one microtubule of each pair and connects the doublet to the center of the structure.

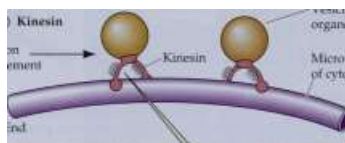
In the cytoplasm at the base of every eukaryotic flagellum or cilium is an organelle called a basal body. The nine microtubule doublets extend into the basal body. In the basal body, each doublet is accompanied by another microtubule, making nine sets of three microtubules. The central, unfused microtubules do not extend into the basal body.

The microtubule doublets of cilia and flagella are linked by proteins. The motion of cilia and flagella results from the sliding of the microtubules past each other, driven by a motor protein called dynein, which can undergo changes

*Some prokaryotes have flagella, as we saw earlier, but prokaryotic flagella lack microtubules and dynein. The flagella of prokaryotes are neither structurally nor evolutionarily related to those of eukaryotes. The prokaryotic flagellum is assembled from a protein called flagellin, and it has a much simpler structure and a smaller diameter than a single eukaryotic microtubule. And whereas eukaryotic flagella beat in a wavelike motion, prokaryotic flagella rotate (see Figure 4.7).

(b) Kinesin

Direction of movement



Vesicle or organelle

Microtubule of cytoskeleton

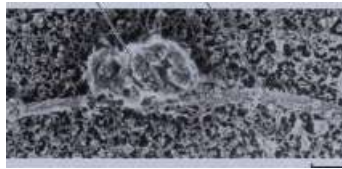
^End

©End

The motor protein kinesin attaches to organelles or vesicles and "walks" them along the microtubules of cytoskeleton. The vesicle moves, while the microtubule is stationary.

Vesicle

Microtubule



25 nm

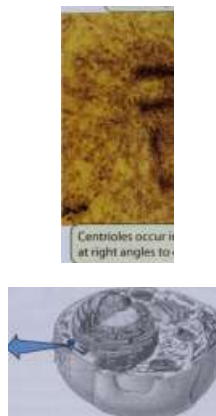
in its shape driven by energy from ATP. Dynein molecules attached to one microtubule bind to a neighboring microtubule. As the dynein molecules change shape, they move the microtubule past its neighbor (Figure 4.25a). Blocking the motor action of dynein is the idea behind a new class of spermicides used for contraception: Because these spermicides inhibit dynein, the sperm cannot swim toward the egg, and fertilization cannot occur.

Dynein and another motor protein, kinesin, are responsible for carrying protein-laden vesicles from one part of the cell to another. Recall that microtubules have a + end and a - end. Dynein binds to a microtubule and moves attached vesicles and other organelles toward the - end, while kinesin moves them toward the + end (Figure 4.25b).

Centrioles are almost identical to basal bodies. Centrioles are found in all eukaryotes except the flowering plants, pine trees and their relatives, and some protists. Under the light microscope, a centriole looks like a small, featureless particle, but the electron microscope reveals that it is made up of a precise bundle of microtubules, arranged as nine sets of three fused microtubules each (Figure 4.26). Centri-

76 CHAPTER FOUR

The structure of a centriole is like that of a cilium's basal body (see Figure 4.25).



Centrioles occur in pairs, at right angles to each other.



0.25 μm

4.26 Centrioles Contain Triplets of Microtubules

Centrioles are found in the microtubule organizing center, a region near the nucleus. The electron micrograph shows a pair of centrioles at right angles to each other.

Centrioles lie in the microtubule organizing center in cells that are about to undergo division. As you will see in Chapter 9, they are involved in the formation of the mitotic spindle, to which the chromosomes attach.

Extracellular Structures

Although the plasma membrane is the functional barrier between the inside and outside of a cell, many structures outside the plasma membrane are produced by cells, secreted to the outside, and play essential roles in protecting, supporting, or attaching cells. These structures are said to be extracellular because they are outside the plasma membrane. The peptidoglycan cell wall of bacteria is such an extracellular structure. In eukaryotes, other extracellular structures play the same roles: in plants, the cellulose cell wall, and in multicellular animals, the extracellular matrix found between cells. Both of these structures are made up of a prominent fibrous macromolecule embedded in a jellylike medium.

The plant cell wall consists largely of cellulose

The cell wall of plant cells is a semirigid structure outside the plasma membrane (Figure 4.27). It consists of cellulose fibers embedded in other complex polysaccharides and proteins. The cell wall has two major roles in plants:

- ▶ It provides support for the cell and limits its volume by remaining rigid.
- ▶ It acts as a barrier to infections by fungi and other organisms that can cause plant diseases.

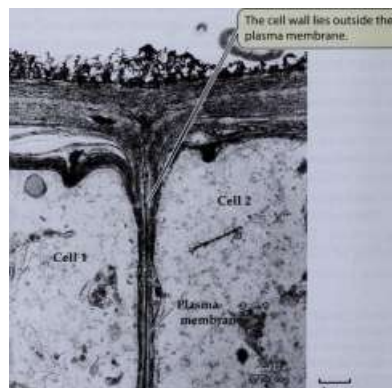
Because of their thick cell walls, plant cells viewed under a light microscope appear to be entirely isolated from each other. But electron microscopy reveals that this is not the

case. The cytoplasm of adjacent plant cells is connected by numerous plasma membrane-lined channels, called plasmodesmata, that are about 20 to 40 nm in diameter and extend through the walls of adjoining cells (see Figure 4.27). These connections permit the diffusion of water, ions, small molecules, and RNA and proteins between connected cells. Such diffusion ensures that the cells of a plant have uniform concentrations of these substances.

Animal cells have elaborate extracellular matrices

The cells of multicellular animals lack the semirigid cell wall that is characteristic of plant cells, but many animal cells are surrounded by, or are in contact with, an extracellular matrix. This matrix is composed of fibrous proteins such as collagen (the most abundant protein in mammals) and glycoproteins (Figure 4.28). These proteins, as well as other substances particular to certain body tissues, are secreted by cells that are present in or near the matrix. In the human body, some tissues, such as those in the brain, have very little extracellular matrix; other tissues, such as bone and cartilage, have large amounts of extracellular matrix. The functions of the extracellular matrix are many:

- ▶ It holds cells together in tissues.
- ▶ It contributes to the physical properties of cartilage, skin, and other tissues.
- ▶ It helps filter materials passing between different tissues.
- ▶ It helps orient cell movements during embryonic development and during tissue repair.
- ▶ It plays a role in chemical signaling from one cell to another.



■ / > ■

', .Cell*

■ '-V

WKKmm iui

4.27 The Plant Cell Wall

The semirigid cell wall provides support for plant cells.

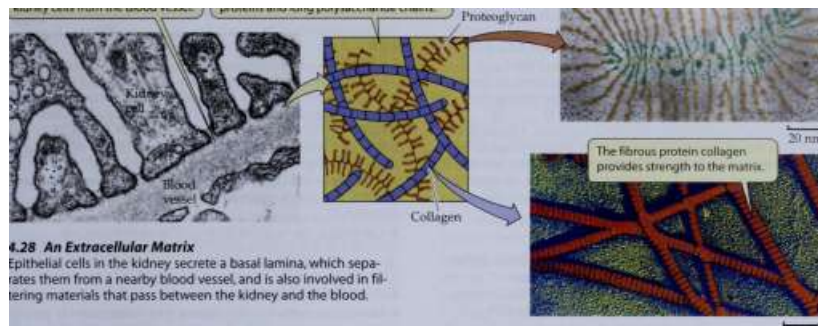
The basal lamina is an extracellular matrix (ECM). Here it separates kidney cells from the blood vessel.

The ECM is composed of a tangled complex of enormous molecules made of proteins and long polysaccharide chains.

THE ORGANIZATION OF CELLS 77

Proteoglycans are long polysaccharide chains that provide a viscous medium for filtering.

H "



4.28 An Extracellular Matrix

Epithelial cells in the kidney secrete a basal lamina, which separates them from a nearby blood vessel, and is also involved in filtering materials that pass between the kidney and the blood.

100 nm

The cells embedded in bone and cartilage, for example, secrete and maintain the extracellular material that makes up these structures. Bone cells are embedded in an extracellular matrix that consists primarily of collagen and calcium phosphate. This matrix gives bone its familiar rigidity. Epithelial cells, which line body cavities, lie together as a sheet spread over a basal lamina, or basement membrane, a form of extracellular matrix (see Figure 4.28).

Some extracellular matrices are made up, in part, of an enormous proteoglycan. A single molecule of this proteoglycan consists of many hundreds of polysaccharides covalently attached to about a hundred proteins, all of which are attached to one enormous polysaccharide. The molecular weight of this proteoglycan can exceed 100 million; the molecule takes up as much space as an entire prokaryotic cell.

Chapter Summary

The Cell: The Basic Unit of Life

- All cells come from preexisting cells and have certain processes, types of molecules, and structures in common.
- To maintain adequate exchanges with its environment, a cell's surface area must be large compared with its volume. Review Figure 4.2
- Microscopes are needed to visualize cells. Because of their greater resolving power, electron microscopes allow observation of greater detail than can be seen with light microscopes. Review Figure 4.3
- Prokaryotic cell organization is characteristic of the kingdoms Eubacteria and Archaeobacteria. Prokaryotic cells lack internal compartments. Review Figure 4.4
- Eukaryotic cell organization is characteristic of cells in the other four kingdoms. Eukaryotic cells have many membrane-enclosed compartments, including a nucleus that contains DNA. Review Figure 4.7

Prokaryotic Cells

- All prokaryotic cells have a plasma membrane, a nucleoid region with DNA, and a cytoplasm that contains ribosomes, dissolved enzymes, water, and small molecules. Some prokaryotes have additional protective structures: cell wall, outer membrane, and capsule. Some prokaryotes contain photosynthetic membranes, and some have mesosomes. Review Figures 4.4, 4.5
- Projecting from the surface of some prokaryotes are rotating flagella, which move the cells from place to place. Pili are projections by which prokaryotic cells attach to one another or to environmental surfaces. Review Figure 4.6

Eukaryotic Cells

- Like prokaryotic cells, eukaryotic cells have a plasma membrane, cytoplasm, and ribosomes. However, eukaryotic cells are larger and contain many membrane-enclosed organelles. Review Figure 4.7
- The membranes that envelop organelles in the eukaryotic cell are partial barriers, ensuring that the chemical composition of the interior of the organelle differs from that of the surrounding cytoplasm.

Organelles that Process Information

- The nucleus is usually the largest organelle in a cell. It is surrounded by a double membrane, the nuclear envelope, which disassembles during cell division. Within the nucleus, the nucleolus is the source of the ribosomes found in the cytoplasm. Review Figure 4.8
- Nuclear pores have complex structures that govern what enters and leaves the nucleus. Review Figure 4.8
- The nucleus contains most of the cell's DNA, which associates with protein to form chromatin. Chromatin is diffuse throughout the nucleus until just before cell division, when it condenses to form chromosomes. Review Figure 4.9

The Endomembrane System

- ▶ The endomembrane system is made up of a series of interrelated membranes and compartments.

78 CHAPTER FOUR

- ▶ The rough endoplasmic reticulum has attached ribosomes that synthesize proteins. The smooth endoplasmic reticulum lacks ribosomes and is associated with the synthesis of lipids. Review Figures 4.7, 4.11
- ▶ The Golgi apparatus adds signal molecules to proteins, directing them to their proper destinations. It receives materials from the rough ER by means of vesicles that fuse with the cis region of the Golgi. Review Figures 4.7, 4.12, 4.13
- ▶ Vesicles originating from the trans region of the Golgi contain proteins for different cellular locations. Some of these vesicles fuse with the plasma membrane and release their contents outside the cell. Review Figure 4.12
- ▶ Lysosomes contain many digestive enzymes. Lysosomes fuse with the phagosomes produced by phagocytosis to form secondary lysosomes, in which engulfed materials are digested. Undigested materials are secreted from the cell when the secondary lysosome fuses with the plasma membrane. Review Figure 4.13

Organelles that Process Energy

- ▶ Mitochondria are enclosed by an outer membrane and an inner membrane that folds inward to form cristae. Mitochondria contain the proteins needed for cellular respiration and the generation of ATP. Review Figure 4.14
- ▶ All eukaryotic cells contain mitochondria. Green plant cells also contain chloroplasts. These organelles are enclosed by double membranes and contain an internal system of thylakoids organized as grana. Review Figures 4.7, 4.16
- ▶ Thylakoids within chloroplasts contain the chlorophyll and proteins that harvest light energy for photosynthesis. Review Figure 4.16
- ▶ Both mitochondria and chloroplasts contain their own DNA and ribosomes and are capable of making some of their own proteins.
- ▶ The endosymbiosis theory of the evolutionary origin of mitochondria and chloroplasts states that these organelles originated when larger prokaryotes engulfed, but did not digest, smaller prokaryotes. Mutual benefits permitted this symbiotic relationship to be maintained and to evolve into the eukaryotic organelles observed today. Review Figure 4.18

Other Organelles Enclosed by Membranes

- ▶ Peroxisomes and glyoxysomes contain special enzymes and carry out specialized chemical reactions inside the cell.
- ▶ Vacuoles are prominent in many plant cells and consist of a membrane-enclosed compartment full of water and dissolved substances. By taking in water, vacuoles enlarge and provide the pressure needed to stretch the cell wall and provide structural support for the plant.

The Cytoskeleton

- ▶ The cytoskeleton within the cytoplasm of eukaryotic cells provides shape, strength, and movement. It consists of three interacting types of protein fibers. Review Figure 4.21

- ▶ Microfilaments consist of two chains of actin units that together form a double helix. Microfilaments strengthen cellular structures and provide the movement in animal cell

division, cytoplasmic streaming, and pseudopod extension. Microfilaments may be found as individual fibers, bundles of fibers, or networks of fibers joined by linking proteins. Review Figures 4.21, 4.23

- ▶ Intermediate filaments are formed of keratins and are organized into tough, ropelike structures that add strength to cell attachments in multicellular organisms. Review Figure 4.21

- ▶ Microtubules are composed of dimers of the protein tubulin. They can lengthen and shorten by adding and losing tubulin dimers. They are involved in the structure and function of cilia and flagella, both of which have a characteristic 9 + 2 pattern of microtubules. Review Figures 4.21, 4.24

- ▶ The movements of cilia and flagella are due to the binding of the motor protein dynein to the microtubules. Microtubules also bind motor proteins, including kinesin and dynein, that move organelles through the cell. Review Figure 4.25

- ▶ Centrioles, made up of triplets of microtubules, are involved in the distribution of chromosomes during nuclear division. Review Figure 4.26

Extracellular Structures

- ▶ Materials external to the plasma membrane provide protection, support, and attachment for cells in multicellular systems.
- ▶ The cell wall of plants consists principally of cellulose. It is pierced by plasmodesmata that join the cytoplasm of adjacent cells. Review Figure 4.27

► In multicellular animals, the extracellular matrix consists of different kinds of proteins, including proteoglycan. In bone and cartilage, the protein collagen predominates. Review Figure 4.28

For Discussion

1. Which organelles and other structures are found in both plant and animal cells? Which are found in plant but not animal cells? In animal but not plant cells? Discuss these differences in relation to the activities of plants and animals.
2. Through how many membranes would a molecule have to pass in going from the interior of a chloroplast to the interior of a mitochondrion? From the interior of a lysosome to the outside of a cell? From one ribosome to another?
3. How does the possession of double membranes by chloroplasts and mitochondria relate to the endosymbiosis theory of the origins of these organelles? What other evidence supports the theory?
4. What kinds of cells and subcellular structures would you choose to examine by transmission electron microscopy? By scanning electron microscopy? By light microscopy? What are the advantages and disadvantages of each of these modes of microscopy?



Cellular Membranes



"No sweat" may describe your reaction to a course with a light workload. But it certainly does not apply to a professional athlete—or to anyone else—who is engaging in vigorous activity. The harder we work physically, the hotter we get, and soon we start to sweat. Sweating is a way to reduce body heat by using the excess heat to evaporate water. At peak activity, we lose as much as 2 liters of water in an hour.

The sweat glands lie just below the surface of the skin. They are essentially tubes bathed in extracellular fluid. When stimulated by physical activity or other signals, these tubes fill with water and dissolved solutes. To get from the extracellular fluid into the tubes, water must pass into and through the cells that line the tube.

A hallmark of living cells is their ability to regulate what enters and leaves their cytoplasm. This is a function of the plasma membrane, which is composed of a hydrophobic lipid bilayer with associated proteins. When a person engages in normal activities, the membranes of the cells lining their sweat glands do not allow much water to enter or leave. But when the same person exercises, special pore proteins in the membrane, called aquaporins, open and allow water from the extracellular fluid to pass through the cells into a tube that leads to the surface of the skin.

Membranes are dynamic structures whose components move, change, and perform vital physiological roles as they allow cells to interact with other cells and molecules in the environment. We describe the structural aspects of these interactions here. Membranes also regulate ionic and molecular traffic into and out of the cell. This selective permeability, which we describe in this chapter, is an important characteristic of life. Later, we will see it in action in such diverse situations as the transduction of light energy into chemical energy in the chloroplast and the retention of water and ions in the mammalian kidney.

Membrane Composition and Structure

The chemical makeup, physical organization, and functioning of a biological membrane depend on three classes of biochemical compounds: lipids, proteins, and carbohy-

Sweating: A Regulated Membrane Activity

Tennis star Venus Williams, shown here winning a gold medal at the 2000 Olympic Games, can lose up to 2 liters of water in an hour by sweating. The excess body heat generated by her physical activity is used to evaporate the sweat, helping to keep her body temperature at normal levels.

drates (Figure 5.1). The lipids establish the physical integrity of the membrane and create an effective barrier to the rapid passage of hydrophilic materials such as water and ions. In addition, the phospholipid bilayer serves as a lipid "lake" in which a variety of proteins "float." This general design is known as the fluid mosaic model of the membrane. Membrane proteins embedded in the phospholipid bilayer have a number of functions, including moving materials through the membrane and receiving chemical signals from the cell's external environment.

Like some proteins, carbohydrates—the third class of compounds important in membranes—are crucial in recognizing specific molecules. The carbohydrates attach either to lipid or to protein molecules on the outside of the plasma membrane, where they protrude into the environment, away from the cell.

Lipids constitute the bulk of a membrane

Nearly all of the lipids in biological membranes are phospholipids. Recall from Chapter 2 that some compounds are hydrophilic ("water-loving") and others are hydrophobic



80 CHAPTER FIVE

("water-hating"). Phospholipids are both: They have both hydrophilic regions and hydrophobic regions. The long, nonpolar fatty acid "tails" of a phospholipid molecule are hydrophobic and associate easily with other nonpolar materials, but they do not dissolve in water or associate with hydrophilic substances. The phosphorus-containing "head" of the phospholipid is electrically charged and hence very hydrophilic.

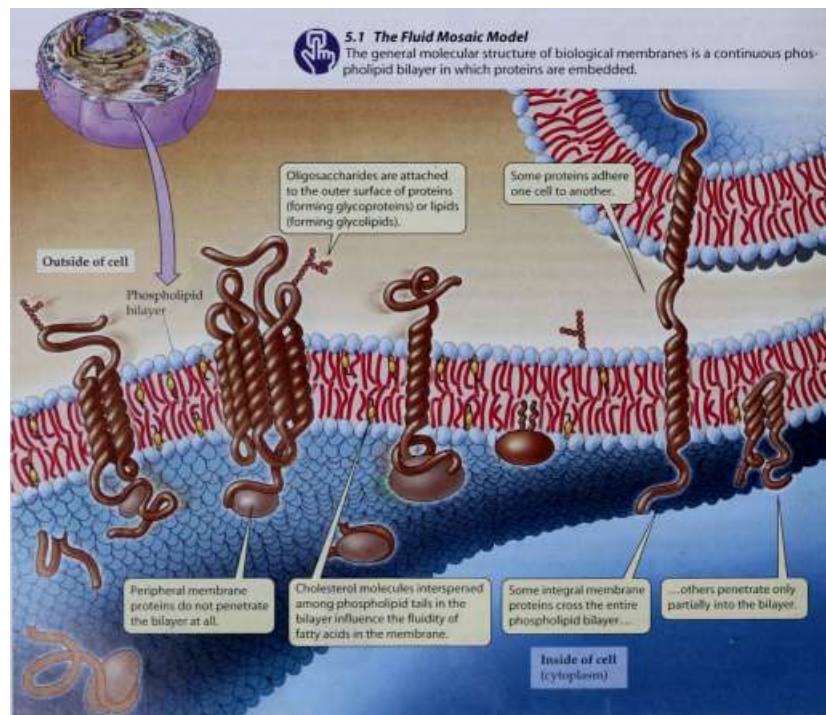
As a consequence of these properties, one way in which phospholipids can coexist with water is to form a double layer, with the fatty acids of the two layers interacting with each other and the polar regions facing the outside aqueous environment (Figure 5.2). It is easy to make artificial membranes with the same bilayered arrangement in the laboratory. Both artificial and natural membranes form continuous sheets. Because of the tendency of the nonpolar fatty acids to associate with one another and exclude water, small holes or rips in a membrane seal themselves spontaneously. This property helps membranes fuse during vesicle fusion, phagocytosis, and related processes.

The phospholipid bilayer stabilizes the entire membrane structure. At the same time, the fatty acids of the phospholipids make the hydrophobic interior of the membrane somewhat fluid—about as fluid as lightweight machine oil. This fluidity permits some molecules to move laterally within the plane of the membrane. A given phospholipid molecule in the plasma membrane may travel from one end of the cell to the other in a little more than a second. On the other hand, seldom does a phospholipid molecule in one half of the bilayer flip over to the other side and trade places with another phospholipid molecule. For such a swap to happen, the polar part of each molecule would have to move through the hydrophobic interior of the membrane. Since phospholipid flip-flops are rare, the inner and outer halves of the bilayer may be quite different in the kinds of phospholipids present.

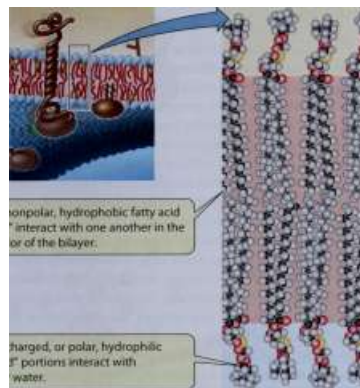
All biological membranes have a similar structure, but membranes from different cells or organelles may differ greatly in their lipid composition. For example, 25% of the lipid in some membranes is cholesterol (see Chapter 3), but

[rn ^ 5.7 The Fluid Mosaic Model

The general molecular structure of biological membranes is a continuous phospholipid bilayer in which proteins are embedded.



Aqueous environment



The nonpolar, hydrophobic fatty acid "tails" interact with one another in the interior of the bilayer.

The charged, or polar, hydrophilic "head" portions interact with polar water.

Aqueous environment

5.2 A Phospholipid Bilayer Separates Two Aqueous Regions

The eight phospholipid molecules shown here represent a small cross section of a membrane bilayer.

Other membranes have no cholesterol at all. When present, cholesterol is important to membrane integrity; most cholesterol in membranes is not hazardous to your health. A molecule of cholesterol is commonly situated next to an unsaturated fatty acid, and the polar hydroxyl end of the cholesterol extends into the surrounding aqueous layer (see Figure 5.1).

Cholesterol may either increase or decrease membrane fluidity, depending on other factors, such as fatty acid composition. Shorter fatty acid chains make for a more fluid membrane, as do unsaturated fatty acids. Adequate membrane fluidity is essential for many membrane functions. Since molecules move more slowly and fluidity decreases at reduced temperatures, membrane functions may decline in organisms that cannot keep their bodies warm. To address this problem, some organisms simply change the lipid compositions of their membranes, replacing saturated with unsaturated fatty acids and using fatty acids with shorter tails. Such changes play a part in the survival of plants and hibernating animals and bacteria during the winter.

Membrane proteins are asymmetrically distributed

All biological membranes contain proteins. Typically, plasma membranes have 1 protein molecule for every 25 phospholipid molecules. This ratio varies, depending on membrane function. In the inner membrane of the mitochondrion, which is specialized for energy processing, there

5.3 Membrane Proteins Revealed by the Freeze-Fracture Technique

This membrane from a spinach chloroplast was first frozen and then separated so that the membrane bilayer was split open.

CELLULAR MEMBRANES 81

is 1 protein for every 15 lipids; myelin, which encloses nerve cells and uses the properties of lipids to act as an electrical insulator, has only 1 protein per 70 lipids.

Many membrane proteins are embedded in, and/or extend across, the lipid bilayer. Like phospholipids, these proteins have polar and nonpolar regions. In the polar regions, amino acids with hydrophilic R groups (side chains) predominate, while the nonpolar regions have amino acids with hydrophobic R groups (see Table 3.2). Like the phospholipids, these proteins are positioned in the membrane so that the polar ends stick out into the aqueous environment and the nonpolar regions aggregate with one another (and with the nonpolar fatty acid tails of the lipids) away from water.

A special preparation method for electron microscopy, freeze-fracturing, reveals membrane proteins embedded in the lipid bilayer (Figure 5.3). The bumps that can be seen protruding from the interior of a membrane are not observed in pure lipid bilayers.

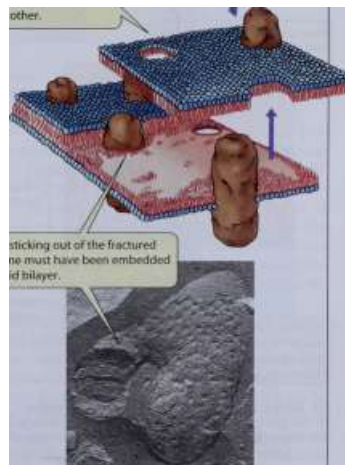
TECHNIQUE

VARIABLES

Frozen tissue is fractured with a knife.



Fracturing causes one half of the membrane to separate from the other.



Proteins sticking out of the fractured membrane must have been embedded in the lipid bilayer.

82 CHAPTER FIVE

According to the fluid mosaic model, the proteins and lipids in a membrane are independent of each other, and interact only noncovalently. The polar ends of proteins can interact with polar ends of lipids, and the nonpolar regions of both molecules interact hydrophobically (see Figure 5.1).

There are two general types of membrane proteins:

- ▶ Integral membrane proteins have hydrophobic regions, and penetrate the phospholipid bilayer. Many of these proteins have long hydrophobic α -helical regions that span the hydrophobic core of the bilayer. Their hydrophilic ends protrude into the aqueous environments on either side of the membrane (Figure 5.4).
- ▶ Peripheral membrane proteins lack hydrophobic regions, and are not embedded in the bilayer. Instead, they have polar or charged regions that interact with similar regions on exposed parts of the integral membrane proteins or phospholipid molecules.

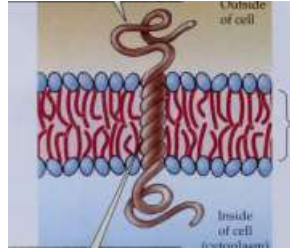
Some membrane proteins are covalently attached to fatty acids or other lipid groups. These proteins can be classified as a special type of integral protein, as their hydrophobic lipid component allows them to insert themselves into the lipid bilayer.

Like the lipids, many membrane proteins move relatively freely within the phospholipid bilayer. Experiments using the technique of cell fusion illustrate this migration dramatically. When two cells are fused, a single continuous membrane forms and surrounds both cells, and some proteins from each cell distribute themselves uniformly around this membrane.

Although many proteins are mobile in the membrane, some are not free to migrate but appear to be "anchored" by components of the cytoskeleton. This anchoring can re-

Hydrophilic R groups in exposed parts of the protein interact with water.

Outside of cell



Aqueous exterior of bilayer

Hydrophobic interior of bilayer

Hydrophobic R groups in this part of the protein interact with the hydrophobic core of the membrane, away from water.

Inside

of cell

(cytoplasm)

Aqueous exterior of bilayer

5.4 Interactions of Integral Membrane Proteins

An integral membrane protein is held in the membrane by the distribution of the hydrophilic and hydrophobic side chains of its amino acids.

suit in a segregation of these proteins, resulting in functional specialization to different regions on the cell surface. For example, in certain muscle cells, the plasma membrane protein that serves as a receptor for the chemical signal from nerve cells is normally found only at the site where a nerve cell meets the muscle cell. None of this protein is found elsewhere on the surface of the muscle cell.

Proteins are asymmetrically distributed on the inner and outer surfaces of a membrane. Transmembrane proteins show different "faces" on the two membrane surfaces. Such proteins have certain specific domains (or regions) of their primary structure on the outer side of the membrane, other domains within the membrane, and still other domains on the inner side of the membrane. Peripheral membrane proteins are localized on one side of the membrane or the other, but not both. This arrangement gives the two surfaces of the membrane different properties. As we will see when we discuss active transport, these differences have great functional significance.

Membrane carbohydrates are recognition sites

In addition to lipids and proteins, all plasma membranes and some internal cytoplasmic membranes contain significant amounts of carbohydrates. The carbohydrates are located on the outer surface of the membrane and serve as recognition sites for other cells and molecules (see Figure 5.1).

Membrane-associated carbohydrates may be covalently bound to lipids or to proteins. A carbohydrate bound to a lipid forms a glycolipid. The carbohydrate units of glycolipids often extend to the outside of the membrane, where they serve as recognition signals for interactions between cells. For example, the carbohydrate of some glycolipids changes when a cell becomes cancerous. This change may allow white blood cells to target the cancer cells for destruction.

Most of the carbohydrate in membranes is covalently bound to proteins, forming glycoproteins. The bound carbohydrates are oligosaccharide chains, usually not exceeding 15 monosaccharide units in length. The chains are added to membrane proteins inside the endoplasmic reticulum and are modified in the Golgi apparatus.

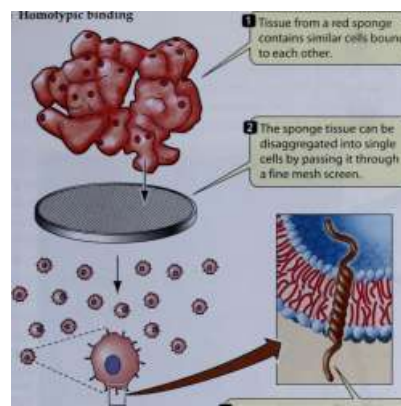
Glycoproteins enable a cell to be recognized by other cells and proteins. An "alphabet" of monosaccharides can be used to generate a diversity of messages. Recall from Chapter 3 that sugar molecules can be formed from 3-7 carbons attached at different sites to one another, forming linear or branched oligosaccharides with many different three-dimensional shapes. An oligosaccharide of a specific shape from one cell can bind to a mirror-image shape on an adjacent cell. This binding forms the basis of cell-to-cell adhesion.

Cell Adhesion

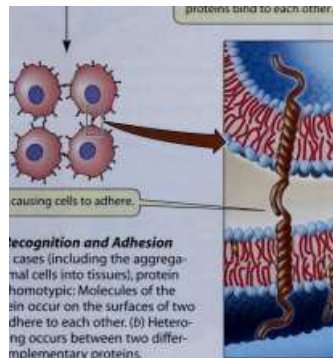
A living sponge is a multicellular marine animal with a simple body plan (see Chapter 31). The cells of the sponge are stuck together, but they can be disaggregated mechanically by passing the animal several times through a fine wire screen. What was an animal is now hundreds of individual of cells, suspended in seawater. Remarkably, if the

(a) Homotypic binding

Tissue from a red sponge contains similar cells bound to each other.



(b) Heterotypic binding



o Exposed regions of membrane

proteins bind to each other...

..causing cells to adhere.

5.5 Cell Recognition and Adhesion

(a) In most cases (including the aggregation of animal cells into tissues), protein binding is homotypic: Molecules of the same protein occur on the surfaces of two cells and adhere to each other. (fc») Heterotypic binding occurs between two different but complementary proteins.

cell suspension is shaken for a few hours, the cells reaggre-gate into a sponge!

If two different species of sponge are disaggregated together, the cells of each species reaggregate into that species only. Such tissue-specific and species-specific cell adhesions are essential in the formation and maintenance of tissues and organisms. Think of the skin in your arm: What keeps its cells together and separates the skin from the underlying bones? You will see many examples of specific cell adhesion throughout this book; here, we describe its general principles.

Cell adhesion involves recognition proteins

The factor responsible for cell-cell recognition and adhesion in sponges was the first such molecule to be identified and purified. It is a huge membrane glycoprotein (80% sugar), partly embedded in the plasma membrane, with the recognition part sticking out and exposed to the environ-



H Mating type

3)

3> ty

OThese gametes from

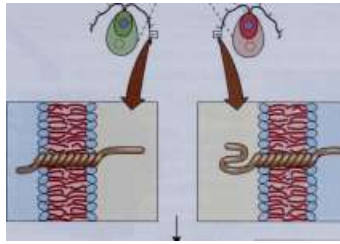
an alga (a marine plant) look ^ identical but have different

v > ^ cell surface proteins.

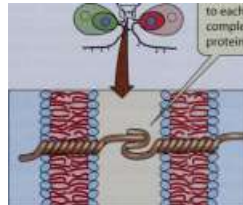
^ ^ ■

#. ^

Mating type



:W_x*



|The gametes adhere to each other by complementary protein binding.

ment (and to other sponge cells). Since then, many such recognition proteins have been purified.

As we saw in Chapter 3, a macromolecule such as a protein has not only a specific shape, but also specific chemical groups. Both features allow' binding to other specific molecules (Figure 5.5). In most cases, the binding of cells in a tissue is homotypic; that is, the same molecule sticks out of both cells, and the exposed surfaces bind to each other. But heterotypic binding (of cells with different proteins) also can occur. For example, when the mammalian sperm meets the egg, different proteins on the two types of cells have complementary binding surfaces.

Plant cells also have recognition proteins. Some single-celled plants form similar-appearing reproductive cells (analogous to male and female) that have flagella to propel them toward each other. Male and female cells can recognize each other by heterotypic proteins on their flagella. In the majority of plant cells, the plasma membrane is covered with a thick cell wall, and this, too, has adhesion proteins that allow cells to bind to one another.

84 CHAPTER FIVE



Plasma membranes

Intercellular space

Junctiona proteins

Tight junctions bar the movement of dissolved materials from the lumen through the space between epithelial cells. There is no intercellular space where there is a tight junction. Long rows of tight-junction proteins form a complex meshwork, seen at the bottom of the freeze-etched image.



(b)

Plasma membranes

Intercellular space

Cytoplasmic" plaque

Connecting fibers

Keratin fibers associated with cytoplasmic plaque

Desmosomes tightly link adjacent cells but permit materials to move around them in the intercellular space. Anchored in dense plaques, cell adhesion proteins cross the intercellular space, binding adjacent cells together. Keratin fibers extend through the cytoplasm from one plaque to another.



[r]LM 5.6 Junctions Link Animal Cells Together

(a,b) Tight junctions and desmosomes are abundant in epithelial tissues, (c) Gap junctions are also found in muscle and nerve tissues.

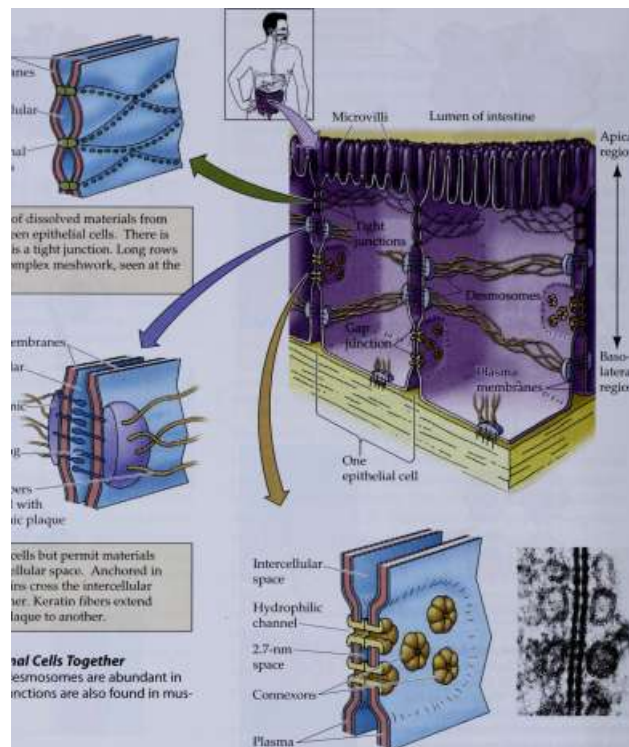
Cell adhesion proteins from many multicellular organisms have been characterized. Some of them do not just bind the two cells together, but initiate the formation of specialized cell junctions.

Specialized Cell Junctions

In a complex multicellular organism, cell-cell recognition proteins allow specific kinds of cells to adhere to each other. Often, both cells contribute material to additional membrane structures that "cement" their relationship. These specialized structures, called cell junctions, are most evident in electron micrographs of epithelial tissues, which are layers of cells that line body cavities or cover body surfaces. We will examine three types of cell surface junctions that enable cells to make direct physical contact and link with one another: tight junctions, desmosomes, and gap junctions

Tight junctions seal tissues and prevent leaks

Tight junctions are specialized structures at the plasma membrane that link adjacent epithelial cells. They result



Plasma

membranes

(c)

Gap junctions let adjacent cells communicate. Dissolved molecules and electric signals may pass from one cell to the other through the channels formed by two connexons extending from adjacent cells.

from the mutual binding of strands of specific membrane proteins that form a series of joints encircling the epithelial cells. They are found in the region surrounding the lumen (cavity) of organs such as the intestine (Figure 5.6a). Tight junctions have two functions:

- They prevent substances from moving through the intercellular space. Thus, any substance entering the body from the lumen must pass through the epithelial cells.
- They restrict the migration of membrane proteins and phospholipids from one region of the cell to another. Thus, the proteins and phospholipids in the region facing the lumen (apical) can be different from those in the regions facing the sides and bottom of the cell (baso-lateral).

By forcing materials to enter some cells, and by allowing different ends of cells to have different membrane proteins with different functions, tight junctions help ensure the directional movement of materials into the body

Desmosomes hold cells together

Desmosomes are specialized structures associated with the plasma membrane at certain sites in epithelial tissues. They hold adjacent cells firmly together, acting like spot welds or rivets (Figure 5.6b). Each desmosome has a dense plaque on the cytoplasmic surface of the plasma membrane. This plaque is attached to fibers in the cytoplasm and special cell adhesion proteins in the plasma membrane. These proteins stretch from the plaque through the plasma membrane of one cell, across the intercellular space, and through the plasma membrane of the adjacent cell, where they bind to the plaque proteins in that cell.

The cytoplasmic fibers of a desmosome, which are intermediate filaments of the cytoskeleton (see Figure 4.21), are made of a protein called keratin. They stretch from one cytoplasmic plaque across the cell to connect with another plaque on the other side of the cell. Anchored thus on both sides of the cell, these extremely strong keratin fibers provide great mechanical stability to epithelial tissues, which often receive rough wear in protecting the organism's body surface integrity.

Gap junctions are a means of communication

Whereas tight junctions and desmosomes have mechanical roles, gap junctions facilitate communication between cells. Each gap junction is made up of specialized protein channels, called connexons, that span the plasma membranes of two adjacent cells and the intercellular space between them (Figure 5.6c). We will describe their role in more detail, as well as that of plasmodesmata, which perform a similar role in plants, when we discuss cell communication in Chapter 15.

©Passive Processes of Membrane Transport

We have examined membrane structure and how it is used to perform one membrane function: the binding of one cell to another. Now we turn to the second major membrane function, selective permeability: the ability to allow some substances, but not others, to pass through the plasma membrane and enter or leave the cell.

There are two fundamentally different processes by which substances cross biological membranes to enter and leave cells or organelles: passive processes and active processes. Passive processes include the different types of diffusion: simple diffusion through the phospholipid bi-layer, and facilitated diffusion through channel proteins or by means of carrier molecules. Active processes, on the other hand, require the input of energy. We'll discuss the active processes later in this chapter, after first focusing on the passive processes. Before considering diffusion as it works across a membrane, however, we must understand the basic principles of diffusion.

The physical nature of diffusion

Nothing in this world is ever absolutely at rest. Everything is in motion, though the motions may be very small. As the temperature of a solution rises, its molecules and ions move faster—they vibrate, rotate, and move from place to place more quickly. An important consequence of this random jiggling is that all the components of a solution tend eventually to become evenly distributed throughout the system. For example, if a drop of ink is allowed to fall into a container of water, the pigment molecules of the ink are initially very concentrated. Without human intervention such as stirring, the pigment molecules of the ink move about at random, spreading slowly through the water until eventually the concentration of pigment—and thus the intensity of color—is exactly the same in every drop of liquid in the container. A solution in which the particles are uniformly distributed is said to be at equilibrium, because there will be no future net change in concentration.

Diffusion is the process of random movement toward a state of equilibrium. Although the motion of each individual particle is absolutely random, in diffusion the net movement of particles is directional until equilibrium is reached. Diffusion is thus net movement from regions of greater concentration to regions of lesser concentration (Figure 5.7).

In a complex solution (one with many different solutes), the diffusion of each substance is independent of that of the others. How fast a substance diffuses depends on four factors: (1) the diameter of the molecules or ions; (2) the temperature of the solution; (3) the electric charge, if any, of the diffusing material; and (4) the concentration gradient in the system. The concentration gradient is the change in concentration with distance in a given direction. The greater the concentration gradient, the more rapidly a substance diffuses.

diffusion within cells and tissues. Within cells, or wherever distances are very short, solutes distribute themselves rapidly by diffusion. Small molecules and ions may move from one end of an organelle to another in a millisecond (10^{-3} s). On the other hand, the usefulness of diffusion as a transport mechanism declines drastically as distances become greater. In the absence of mechanical stirring, diffusion across more than a centimeter may take an hour or more, and diffusion across meters may take years! Diffusion would not be adequate to distribute materials over the length of the human body, but within our cells or across layers of one or two cells, diffusion is rapid enough to distribute small molecules and ions almost instantaneously.

diffusion across membranes. In a solution without barriers, all the solutes diffuse at rates determined by temperature, their physical properties, and the concentration gradient of each solute. If a biological membrane is introduced as a barrier, the movement of the different solutes can be affected by the properties of the membrane. The membrane is said to be permeable to solutes that can cross it more or less easily, but impermeable to substances that cannot move across it. Molecules to which the membrane is permeable

Question: Does diffusion lead to uniform distribution of solutes?

Add equal amounts of three dyes to still water in a shallow container.

Sample different regions of the solution and measure the amount of each colored dye.

The number and position of molecules of each dye can be rendered visually.



1

I

5 minutes later

10 minutes later

J



.....%.....,,.....

Conclusion: Solutes distribute themselves by diffusion, uniformly and independently of each other.

5.7 Diffusion Leads to Uniform Distribution of Solutes

Diffusion is the net movement of a solute from regions of greater concentration to regions of lesser concentration. The speed of diffusion varies with the substances involved, but the process continues until the solution reaches equilibrium.

diffuse from one compartment to the other until their concentrations are equal on both sides of the membrane. Molecules to which the membrane is impermeable remain in separate compartments, and their concentrations remain different on the two sides of the membrane. Equilibrium is reached when the concentrations of the diffusing substance are identical on both sides of the permeable membrane. Individual molecules are still passing through the membrane when equilibrium is established, but equal numbers of molecules are moving in each direction, so there is no net change in concentration.

Simple diffusion takes place through the membrane bilayer

In simple diffusion, small molecules pass through the lipid bilayer of the membrane. The more lipid-soluble the molecule, the more rapidly it diffuses through the bilayer. This statement holds true over a wide range of molecular weights. Only water and the smallest of molecules seem to deviate from this rule, passing through bilayers much more rapidly than their lipid solubilities would predict.

Charged and/or polar molecules such as amino acids, sugars, and ions do not pass readily through a membrane, for two reasons. First, cells are made up of, and exist in, water, and polar or charged substances form many hydrogen bonds with water, preventing their "escape" to the membrane. Second, the interior of the membrane is hy-

drophobic, and hydrophilic substances tend to be excluded from it. On the other hand, a molecule that is itself hydrophobic, and hence soluble in lipids, enters the membrane readily and is thus able to pass through it.

Osmosis is the diffusion of water across membranes

Water molecules are abundant enough and small enough that they move through membranes by a diffusion process called osmosis. This completely passive process uses no metabolic energy and can be understood in terms of the concentrations of solutions. Osmosis depends on the number of solute particles present—not the kind of particles. We will describe osmosis using red blood cells and plant cells as examples.

Red blood cells are normally suspended in a fluid called plasma, which contains salts, proteins, and other solutes. If a drop of blood is examined under the light microscope, the red cells are seen to have their characteristic donut shape. If pure water is added to the drop of blood, the cells quickly swell and burst (Figure 5.8a). Similarly, if slightly wilted lettuce is put in pure water, it soon becomes crisp; by weighing it before and after, we can show that it has taken up water (Figure 5.8b).

If, on the other hand, red blood cells or crisp lettuce leaves are placed in a relatively concentrated solution of salt or sugar, the leaves become limp (wilt) and the red blood cells pucker and shrink. From analyses of such observations, we know that the difference in solute concentrations is the principal factor that determines whether water will move from the surrounding environment into cells, or out of cells into the environment.

CELLULAR MEMBRANES 87

Hypertonic

(concentrated salt solution)

H₂O

Isotonic

(normal salt concentration)

(a) Animal cell (red blood cell)

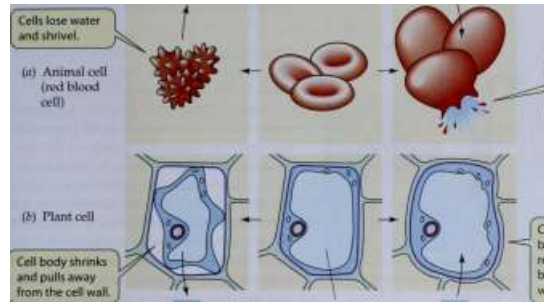
(b) Plant cell

Cell body shrinks and pulls away from the cell wall.

Hypotonic

(distilled water)

H₂O



5.8 Osmosis Modifies the Shapes of Cells

In an isotonic solution (center), plant and animal cells maintain consistent, characteristic shapes. In a hypotonic solution, water enters the cell; a hypertonic environment draws water out of the cell.

Cells take up water, swell, and burst.

Cell stiffens but generally retains its shape because cell wall is present.

H₂O

Vacuole

H₂O

Other things being equal, if two different solutions are separated by a membrane that allows water, but not solutes, to pass through, water molecules will move across the membrane toward the solution with a higher solute concentration. In other words, water will diffuse from a region of its higher concentration (lower concentration of solutes) to a region of its lower concentration (higher concentration of solutes).

Three terms are used to compare the solute concentrations of two solutions separated by a membrane:

- Isotonic solutions have equal total solute concentrations.
- A hypertonic solution has a higher total solute concentration than the other solution with which it is being compared.
- A hypotonic solution has a lower total solute concentration than the other solution with which it is being compared.

Water moves from a hypotonic solution across a membrane to a hypertonic solution.

When we say that "water moves," bear in mind that we are referring to the net movement of water. Since it is so abundant, water is constantly moving across the plasma membrane into and out of cells. Whether the overall movement is greater in one direction or the other is what concerns us here.

The concentration of solutes in the environment determines the direction of osmosis in all animal cells. A red blood cell takes up water from a solution that is hypotonic to the cell's contents. The cell bursts because its plasma membrane cannot withstand the swelling of the cell (see Figure 5.8a). The integrity of red blood cells (and other blood cells) is absolutely dependent on the maintenance of a constant solute concentration in the plasma in which they are suspended: The plasma must be isotonic with the cells if the cells are not to burst or shrink.

In contrast to animal cells, the cells of plants, archaea, bacteria, fungi, and some protists have cell walls that limit the volume of the cells and keep them from bursting. Cells with sturdy cell walls take up a limited amount of water and, in so doing, build up internal pressure against the cell wall that prevents further water from entering. This pressure within the cell, called turgor pressure, is the driving force for the enlargement of plant cells—it is a normal and essential component of plant development.

Diffusion may be aided by channel proteins

As we saw earlier, polar substances such as amino acids and sugars and charged substances such as ions do not diffuse across membranes. Instead, they cross the hydrophobic lipid barrier through protein-lined channels in a process called facilitated diffusion. Integral membrane proteins form these channels (Figure 5.9), which are lined with polar amino acids and water on the inside (to bind to the polar or charged substance and allow it to pass) and nonpolar amino acids on the outside (to allow the protein channel to insert itself into the lipid bilayer).

The best-studied protein channels are the ion channels. As you will see, the movement of ions into and out of cells is important in many biological processes, ranging from the electrical activity of the nervous system to the opening of pores in leaves that allow gas exchange with the environment. Hundreds of these channels have been identified, and all show the basic structure of a water-lined pore that just fits the ion that moves through it.

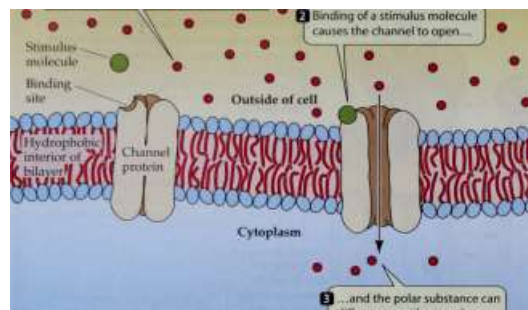
Ion channels are gated: they can be closed to ion passage, or open. A gated channel opens when something happens to change the shape of the protein. Depending on the channel, this stimulus can range from the binding of a chemical signal to an electrical charge caused by an imbalance of ions. Once the channel opens, millions of ions can rush through it

88 CHAPTER FIVE

f

A polar substance is more concentrated on the outside than the inside of the cell.

Stimulus molecule



f

Binding of a stimulus molecule causes the channel to open...

5.9 A Gated Channel Protein Opens in Response to a Stimulus

The membrane protein changes its three-dimensional shape when the stimulus binds.

f

...and the polar substance can diffuse across the membrane.

per second. How fast this happens, and in which direction (into or out of the cell), depends on the concentration gradient of the ion between the cytoplasm and the exterior environment of the cell. For example, if the concentration of potassium ion is much higher outside of the cell than inside, potassium will enter the cell through a potassium channel by diffusion; if it is higher inside the cell, potassium ion will diffuse out of the cell.

As we mentioned, water crosses the plasma membrane at a rate far in excess of expectations, given its polarity. One way that water can do this is by hydrating ions as they pass through ion channels. Up to 12 water molecules may coat an ion as it traverses a channel. Another way that water enters cells rapidly is through water channels called aquaporins. Membrane proteins that allow water to pass through them have been characterized in many cells, from the plant vacuole, where they are important in maintaining turgor, to the mammalian kidney, where they act in retaining water that would otherwise be lost through urine.

favoring glucose entry, with a higher concentration outside the cell (in blood capillaries or the intestine) than inside.

Transport by carrier proteins is different from simple diffusion. In both processes, the rate of movement depends on the concentration gradient across the membrane. However, in facilitated diffusion, a point is reached at which further increases in the concentration gradient are not accompanied by an increased rate of diffusion. At this point, the facilitated diffusion system is said to be saturated. Because there are only a limited number of carrier protein molecules per unit of membrane area, the rate of movement reaches a maximum when all the carrier molecules are fully loaded with solute molecules. In other words, when the differences in solute concentration across the membrane are sufficiently high, not enough carrier molecules are free at a given moment to handle all the solute molecules.

rTI



Active Transport

In many biological situations, an ion or molecule must be moved across a membrane from a region of lower concentration to a region of higher concentration. In these cases,

5.10 A Carrier Protein Facilitates Diffusion

The carrier protein allows glucose to enter the cell at a faster rate than would be possible by simple diffusion across the membrane barrier.

Carrier proteins aid diffusion by binding substances

Another kind of facilitated diffusion involves not just the opening of a channel, but the actual binding of the transported substance to a membrane protein. These proteins are called carriers, and, like channel proteins, they allow diffusion both into and out of the cell. They are used to transport polar molecules such as sugars and amino acids.

Glucose, for example, is the major energy source for most mammalian cells, and those cells have a carrier protein called the glucose transporter that facilitates the uptake of glucose (Figure 5.10). Since glucose is rapidly altered as soon as it gets into a cell, there is almost always a strong concentration gradient

Outside

of cell ;

The carrier protein

Glucose

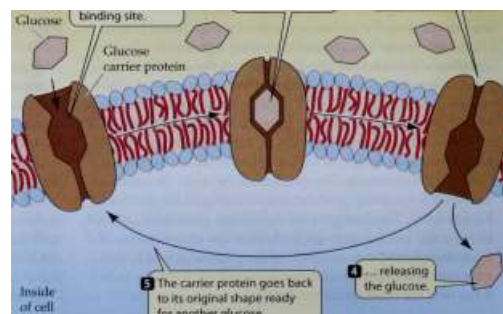
has a glucose binding site.

f

Glucose binds to the protein...



...which then changes its shape...



Inside of cell

| The carrier protein goes back to its original shape ready for another glucose.

CELLULAR MEMBRANES 89

Membrane Transport Mechanisms

SIMPLE DIFFUSION

FACILITATED DIFFUSION

ACTIVE TRANSPORT

Direction

Energy source Membrane protein

required? Specificity

With concentration gradient

Concentration gradient

No

Not specific

With concentration gradient

Concentration gradient Yes

Specific

Against concentration

gradient ATP hydrolysis (primary) Yes

Specific

the substance cannot not rush into or out of cells by diffusion. The movement of a substance across a biological membrane against a concentration gradient—called active transport —requires the expenditure of energy. The differences between diffusion and active transport are summarized in Table 5.1.

Active transport is directional

Three types of proteins are involved in active transport (Figure 5.11):

► Uniport transporters move a single solute in one direction. For example, a Ca^{2+} -binding protein found in the plasma membrane and endoplasmic reticulum membranes of many cells actively transports this ion to regions of higher concentration either outside the cell or inside the ER.

► Symport transporters move two solutes in the same direction. For example, the uptake of amino acids from the intestine into the cells that line it requires the simultaneous binding of Na^+ and amino acid to the same carrier protein.

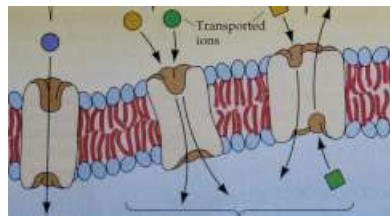
► Antiport transporters move two solutes in opposite directions, one into the cell and the other out of the cell. For example, many cells have an " $\text{Na}^+ - \text{K}^+$ pump" that moves Na^+ out of the cell and K^+ into it.

Uniport transports one substance in one direction.

Symport transports two different substances in the same direction.

Antiport transports two different substances in opposite directions.

^^Transported V ions ■



Coupled transport

5.7 7 Proteins for Active Transport

Note that in each of the three cases, transport is directional.

Primary and secondary active transport rely on different energy sources

There are two basic types of active transport processes. The first, primary active transport, requires the direct participation of ATP. Energy released by the hydrolysis of ATP drives the movement of specific ions against a concentration gradient. For example, if we compare the concentrations of potassium ions (K^+) and sodium ions (Na^+) inside a nerve cell and in the fluid bathing the nerve (Table 5.2), we can see that the K^+ concentration is much higher inside the cell, whereas the Na^+ concentration is much higher outside. Nevertheless, a protein in the nerve cells continues to pump Na^+ out and K^+ in, against these concentration gradients, ensuring that the gradients are maintained. This sodium-potassium pump is found in all animal cells and is an integral membrane glycoprotein. It breaks down a molecule of ATP to ADP and phosphate (P_i), and uses the energy released to bring two K^+ ions into the cell and export three Na^+ ions (Figure 5.12). The $\text{Na}^+ - \text{K}^+$ pump is thus an antiport transport system.

Only cations are transported directly by pumps in primary active transport. Other solutes are transported by secondary active transport. This form of active transport does not use ATP directly; rather, the transport of the solute is tightly coupled to an ion concentration gradient established by primary active transport. The movement of the solute against its concentration gradient is accomplished using energy "regained" by letting ions move across the membrane with their concentration gradient.

For example, energy from ATP is used in primary active transport to establish concentration gradients of potassium and sodium ions; then the passive diffusion of some sodium ions in the opposite direction provides energy for the secondary active transport of the sugar glucose (Figure 5.13). Other secondary active transporters aid in the uptake

50 Concentration of Major Ions Inside • ^ and Outside the Nerve Cell of a Squid

CONCENTRATION (MOLAR)

ION

INSIDE

OUTSIDE

Na +

ci-

0.400 0.050 0.120

0.020 0.440 0.560

03Na + and 1 ATP molecule bind to protein "pump."

f

ADP is released, causing a change in the pump's shape.

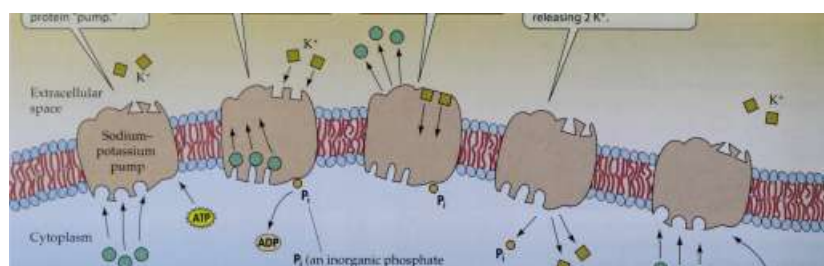
t

3 Na + are released as 2 K + bind to pump.

Q P_i is released, causing the pump's shape to change, and releasing 2 K + .

i The process repeats

3



Cytoplasm

o o o

3Na +

P_i; (an inorganic phosphate ion derived from ATP)

o o o

3Na +

5. 12 Primary Active Transport: The Na + -K + Pump

In active transport, energy is used to move a solute against its concentration gradient. Even though the Na + concentration is higher outside the cell and the K + concentration is higher inside the cell, for each molecule of ATP used, two K + are pumped into the cell and three Na + are pumped out of the cell.

of amino acids and other sugars, which are essential raw materials for cell maintenance and growth. Both types of coupled transport proteins—symports and antiports—are used for secondary active transport.

Endocytosis and Exocytosis

Macromolecules such as proteins, polysaccharides, and nucleic acids are simply too large and too charged or polar to pass through membranes. This is a fortunate property. Think of the consequences if these molecules could diffuse out of cells: A red blood cell would not retain its hemoglobin! On the other hand, cells must sometimes take up or secrete intact large molecules. As we saw in Chapter 4, this occurs by means of vesicles that either pinch off from the plasma mem-

brane and enter the cell (endocytosis) or fuse with the plasma membrane and release their contents (exocytosis).

Macromolecules and particles enter the cell by endocytosis

Endocytosis is a general term for a group of processes that bring macromolecules, large particles, small molecules, and even small cells into the eukaryotic cell (Figure 5.14a). There are three types of endocytosis: phagocytosis, pinocytosis, and receptor-mediated endocytosis. In all three, the plasma membrane invaginates (folds inward) around materials from the environment, forming a small pocket. The pocket deepens, forming a vesicle. This vesicle separates from the surface of the cell and migrates with its contents to the cell's interior.

5.73 Secondary Active Transport

The sodium ion concentration gradient established by primary active transport (right) powers the secondary active transport of glucose (left). The movement of glucose across the membrane against its concentration gradient is coupled by a symport protein to the movement of Na^+ into the cell.

Secondary active transport

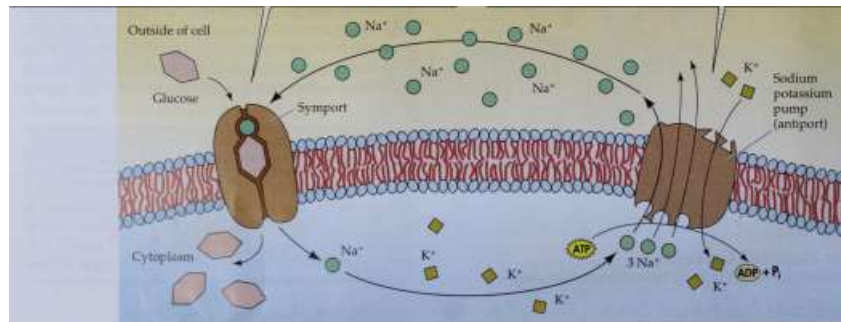
Sodium ions, moving with the concentration gradient established by the sodium-potassium pump, drive transport of glucose against its concentration gradient.

Primary active transport

The sodium-potassium pump moves sodium ions, using the energy of ATP hydrolysis to establish a concentration gradient of Na^+ .

<^> Sodium

O potassium pump (antiport)



(a) Endocytosis

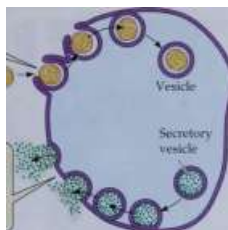
The plasma membrane surrounds a part of the exterior environment and buds off as a vesicle.

Plasma membrane

/

(b) Exocytosis

A vesicle fuses with the plasma membrane. The contents of the vesicle are released, and its membrane becomes part of the plasma membrane.



5.74 Endocytosis and Exocytosis

Endocytosis and exocytosis are used by all eukaryotic cells to take up substances from and release substances to the outside environment.

In phagocytosis, part of the plasma membrane engulfs fairly large particles or even entire cells. Phagocytosis is used as a cellular feeding process by unicellular protists and by some white blood cells that defend the body against foreign cells and substances. The food vacuole or phagosome formed usually fuses with a lysosome, and its contents are digested (see Figure 4.13b).

In pinocytosis ("cellular drinking"), vesicles also form. However, these vesicles are smaller, and the process operates to bring in small dissolved substances or fluids. It is relatively nonspecific as to what it brings into the cell. For example, pinocytosis goes on constantly in the endothelium, the single layer of cells that separates a tiny blood capillary from its surrounding tissue

(Figure 5.15), and is a way for the cells to rapidly acquire the fluids of the blood.

In receptor-mediated endocytosis, specific reactions at the cell surface trigger the uptake of specific materials. Let's take a closer look at this process.

Receptor-mediated endocytosis is highly specific

Receptor-mediated endocytosis is used by animal cells to capture specific macromolecules from the cell's environment. The uptake process is similar to nonspecific endocytosis, as already described. However, in receptor-mediated endocytosis, receptor proteins at particular sites on the outer surface of the plasma membrane bind to specific substances in the environment outside the cell. These sites are called coated pits because they form a slight depression in the plasma membrane whose cytoplasmic surface is coated by fibrous proteins, such as clathrin.

When a receptor protein binds to its specific macromolecule outside the cell, its coated pit invaginates and forms a coated vesicle around the bound macromolecule. Strengthened and stabilized by clathrin molecules, this vesicle carries the macromolecule into the cell (Figure 5.16). Once inside, the vesicle loses its clathrin coat and may fuse with a lysosome, where the engulfed material is processed and released into the cytoplasm. Because of its specificity for particular macromolecules, receptor-mediated endocytosis is a rapid and efficient method of taking up what may be minor constituents of the cell's environment.

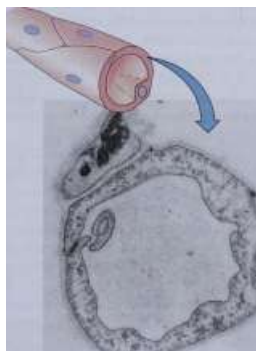
CELLULAR MEMBRANES 91

Receptor-mediated endocytosis is the method by which cholesterol is taken up by most mammalian cells. Water-insoluble cholesterol is synthesized in the liver and transported in the blood attached to a protein, forming a lipoprotein called low-density lipoprotein, or LDL. The uptake of cholesterol begins with the binding of LDL to specific receptor proteins in coated pits. After being engulfed by endocytosis, the LDL particle is freed from the receptors. The receptors segregate to a region of the vesicle that buds off to form a new vesicle, which is recycled to the plasma membrane. The freed LDL particle remains in the original vesicle, which fuses with a lysosome in which the LDL is digested and the cholesterol made available for cell use. Persons with the inherited disease hypercholesterolemia (-emia, "blood") have dangerously high levels of cholesterol in their blood because of a deficient receptor for LDL.

Exocytosis moves materials out of the cell

Exocytosis is the process by which materials packaged in vesicles are secreted from a cell when the vesicle membrane fuses with the plasma membrane (see Figure 5.14fr). The initial event in this process is the binding of a membrane protein protruding from the cytoplasmic side of the vesicle with a membrane protein on the cytoplasmic side of the target site on the plasma membrane. The phospholipid regions of the two membranes merge, and an opening to the outside of the cell develops. The contents of the vesicle are released to the environment, and the vesicle membrane is smoothly incorporated into the plasma membrane.

In Chapter 4, we encountered exocytosis as the last step in the processing of material engulfed by phagocytosis: the secretion of indigestible materials to the environment. Exocytosis is also important in the secretion of many different sub-

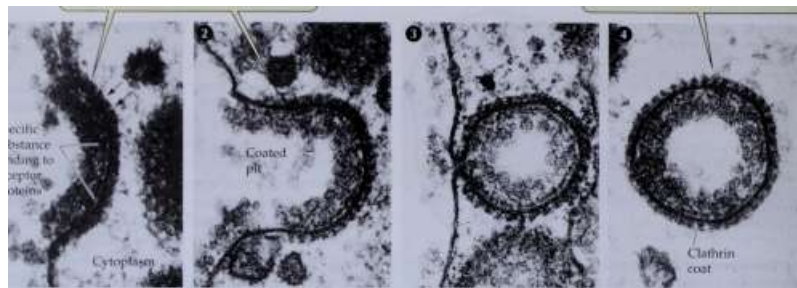


5.75 Pinocytosis and Exocytosis

The single endothelial cell that surrounds a blood capillary uses pinocytosis and exocytosis to transport substances between the blood and the surrounding tissue.

The protein clathrin coats the cytoplasmic side of the plasma membrane at a coated pit.

The endocytosed contents are surrounded by a clathrin-coated vesicle.



Specific substance

binding to receptor proteins

5.16 Formation of a Coated Vesicle

In receptor-mediated endocytosis, the receptor proteins in a coated pit bind specific macromolecules, which are then carried into the cell by the coated vesicle.

stances, including digestive enzymes from the pancreas, neurotransmitters from nerve cells, and materials for the construction of the plant cell wall.

Membranes Are Not Simply Barriers

We have discussed several functions of membranes—the compartmentalization of cells, the regulation of traffic between compartments, and the movement of materials into and out of cells—but there are more. In Chapter 4, we described how the membrane of the rough endoplasmic reticulum serves as a site for ribosome attachment. Newly formed proteins are passed from the ribosomes through the membrane and into the interior of the ER for modification and delivery to other parts of the cell. On the other hand, the membranes of nerve cells, muscle cells, some eggs, and other cells are electrically excitable. In nerve cells, the plasma membrane is the conductor of the nerve impulse from one end of the cell to the other.

Numerous other biological activities and properties discussed in the chapters to follow are associated with membranes. We review three of these here.

information processing. As we have seen, the plasma membranes at cell surfaces and the membranes within cells may have protruding integral membrane proteins or attached carbohydrates that can bind to specific substances in the environment. The binding of a specific substance can serve as a signal to initiate, modify, or turn off a cell function (Figure 5.17a).

In this type of information processing, specificity in binding is essential. We have already seen the role of a specific receptor protein in the endocytosis of LDL and its cargo of cholesterol (see Figure 5.17). Another example is the binding of a hormone, such as insulin, to specific receptors on a target cell, such as a liver cell, to elicit a response—in this case, the uptake of glucose. There are many other examples, which we will discuss in Chapter 15.

0.1 pm

energy transformation. In a variety of cells, the membranes of organelles are specialized for processing energy (Figure 5.17b). For example, the inner mitochondrial membrane helps convert the energy of fuel molecules to the energy in ATP, and the thylakoid membranes of chloroplasts participate in the conversion of light energy to the energy of chemical bonds. The two characteristics of membranes that enable them to participate in these processes are their structural organization and their separation of electric charges.

organizing chemical reactions. Many processes in cells depend on a series of enzyme-catalyzed reactions in which the products of one reaction serve as the reactants for the next. For such a reaction to occur, all the necessary molecules must come together. In a solution, the reactants and enzymes are all randomly distributed, and collisions among them are random. For this reason, a complete series of chemical reactions in solution may occur very slowly. However, if the different enzymes are bound to a membrane in sequential order, the product of one reaction can be released close to the enzyme for the next reaction. With such an "assembly line," reactions proceed more rapidly and efficiently (Figure 5.17c).

Membranes Are Dynamic

As we have seen in this chapter, membranes participate in numerous physiological and biochemical processes. Membranes are dynamic in another sense as well: They are constantly forming, transforming from one type to another, fusing with one another, and breaking down.

In eukaryotes, phospholipids are synthesized on the surface of the smooth endoplasmic reticulum and rapidly distributed to membranes throughout the cell as vesicles form from the ER, move away, and fuse with other organelles. Membrane proteins are inserted into the rough endoplasmic reticulum as they form on ribosomes. Functioning membranes also move about within eukaryotic cells. For example, portions of the rough ER bud away from the ER and join the cis faces of the Golgi apparatus (see Chapter 4). Rapidly—often in less than an hour—these segments of membrane

Information processing

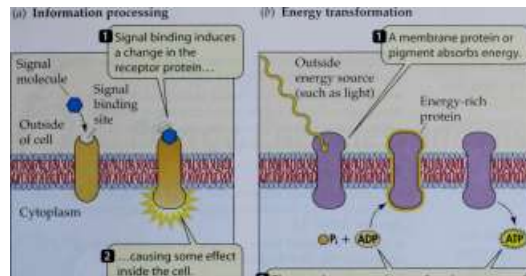
Energy transformation

Signal molecule

\

If Signal binding induces a change in the receptor protein...

A membrane protein or pigment absorbs energy.



Signal ^ binding

Outside \ site of cell AjA

CELLULAR MEMBRANES 93

5.7 7 More Membrane Functions

(a) Membrane proteins conduct signals from outside the cell that trigger changes inside the cell, (b) The membranes of organelles such as mitochondria and chloroplasts are specialized for the transformation of energy, (c) When a series of biochemical reactions must take place in sequence, the membrane can sometimes arrange the enzymes in an "assembly line" to ensure that the reactions occur in proximity to each other.

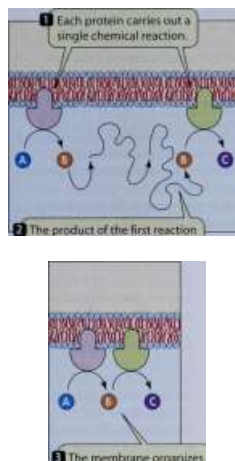
f

...causing some effect inside the cell.

<

The membrane protein transfers the energy to ADP to form ATP, where it is in a form for use by the cell.

(c) Organizing chemical reactions



I The product of the first reaction must diffuse to reach the site of the second reaction.

I The membrane organizes the two reactions so that they occur in the same time and place.

find themselves in the trans regions of the Golgi, from which they bud away to join the plasma membrane (Figure 5.18)

During this journey, changes in the membrane's proteins and phospholipids occur. Membrane from vesicles is constantly merging with the plasma membrane by exocytosis, but this process is largely balanced by the removal of membrane in endocytosis, affording a recovery path by which internal membranes are replenished. In sum, there is a steady flux of membranes and membrane components in cells.

Because all membranes appear similar under the electron microscope, and because they interconvert readily, we might expect all subcellular membranes to be chemically identical. However, that is not the case, for there are major chemical differences among the membranes of even a single cell. Membranes are changed chemically when they form parts of certain organelles. In the Golgi apparatus, for example, the membranes of the cis face closely resemble those of the endoplasmic reticulum in chemical composition, but the trans-face membranes are more similar to the plasma membrane. As a vesicle is

formed, the mix of pro-

5.18 Dynamic Continuity of Cellular Membranes

Membranes continually form, move, and fuse in cells.

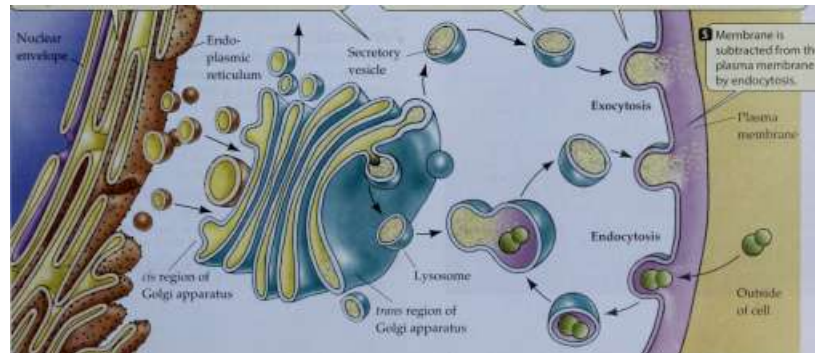
I New stretches of membrane may be generated at certain locations, such as the endoplasmic reticulum.

I Vesicles budding from the trans region of the Golgi apparatus are also membrane-enclosed.

I The vesicles may remain inside the cell as organelles, such as lysosomes...

I.. or they may fuse with the plasma membrane, delivering their contents to the exterior of the cell (exocytosis) and adding their membranes to the plasma membrane.

Membrane is subtracted from the plasma membrane by endocytosis.



Outside of cell

94 CHAPTER FIVE

teins and lipids in its membrane is selected, just as its internal contents are selected, to correspond with the vesicle's target membrane.

Ceaselessly moving, functioning, changing their composition and roles, biological membranes are central to life.

Chapter Summary

Membrane Composition and Structure

- Biological membranes consist of lipids, proteins, and carbohydrates. The fluid mosaic model of membrane structure describes a phospholipid bilayer in which membrane proteins can move about laterally within the membrane. Review Figures 5.1, 5.2
- Integral membrane proteins are at least partially inserted into the phospholipid bilayer. Peripheral proteins attach to the surface of the bilayer by ionic bonds. Review Figure 5.1
- The two surfaces of a membrane may have different properties because of their different phospholipid composition, exposed domains of integral membrane proteins, and their peripheral membrane proteins. Review Figures 5.1, 5.2
- Carbohydrates attached to proteins or phospholipids project from the external surface of the plasma membrane and function as recognition signals for interactions between cells. Review Figure 5.1

Cell Adhesion

- In an organism or tissue, cells recognize and bind to each other by means of membrane proteins that protrude from the cell surface. Review Figure 5.5
- Tight junctions prevent the passage of molecules through the space around cells, and they define functional regions of the plasma membrane by restricting the migration of membrane proteins uniformly over the cell surface. Desmosomes allow cells to adhere strongly to one another. Gap junctions provide channels for chemical and electrical communication between adjacent cells. Review Figure 5.6

Passive Processes of Membrane Transport

- Substances can diffuse passively across a membrane by three processes: unaided diffusion through the phospholipid bilayer, facilitated diffusion through protein channels, or facilitated diffusion by means of a carrier protein. Review Table 5.1
- Solutes diffuse across a membrane from a region with a greater solute concentration to a region with a lesser solute concentration. Equilibrium is reached when the concentrations of a given solute are identical on both sides of the membrane. Review Figure 5.7

- ▶ The rate of simple diffusion of a solute across a membrane is directly proportional to the concentration gradient across the membrane. An important factor in simple diffusion across a membrane is the lipid solubility of the solute.
- ▶ In osmosis, water diffuses from regions of higher water concentration to regions of lower water concentration.
- ▶ In hypotonic solutions, cells tend to take up water, while in hypertonic solutions, cells tend to lose water. Animal cells must remain isotonic to the environment to prevent destructive loss or gain of water. Review Figure 5.8a
- ▶ The cell walls of plants and some other organisms prevent the cells from bursting under hypotonic conditions. The turgor pressure that develops under these conditions keeps plants upright and stretches the cell wall during plant cell growth. Review Figure 5.8b
- ▶ Channel proteins and carrier proteins function in facilitated diffusion. Review Figures 5.9, 5.10
- ▶ The rate of carrier-mediated facilitated diffusion reaches a maximum when a solute concentration is reached that saturates the carrier proteins so that no increase in rate is observed with further increases in solute concentration.

Active Transport

- ▶ Active transport requires the use of energy to move substances across a membrane against a concentration gradient. Review Table 5.1
- ▶ Active transport proteins may be uniports, symports, or antiports. Review Figure 5.11
- ▶ In primary active transport, energy from the hydrolysis of ATP is used to move ions into or out of cells against their concentration gradients. Review Figure 5.12
- ▶ Secondary active transport couples the passive movement of one solute with its concentration gradient to the movement of another solute against its concentration gradient. Energy from ATP is used indirectly to establish the concentration gradient that results in the movement of the first solute. Review Figure 5.13

Endocytosis and Exocytosis

- ▶ Endocytosis transports macromolecules, large particles, and small cells into eukaryotic cells by means of engulfment by and vesicle formation from the plasma membrane. Phagocytosis and pinocytosis are both nonspecific types of endocytosis. Review Figures 5.14, 5.15
- ▶ In receptor-mediated endocytosis, a specific membrane receptor binds to a particular macromolecule. Review Figure 5.16
- ▶ In exocytosis, materials in vesicles are secreted from the cell when the vesicles fuse with the plasma membrane. Review Figure 5.14

Membranes Are Not Simply Barriers

- ▶ Membranes function as sites for recognition and initial processing of extracellular signals, for energy transformations, and for organizing chemical reactions. Review Figure 5.17

Membranes Are Dynamic

- ▶ Although not all cellular membranes are identical, ordered modifications in membrane composition accompany the conversions of one type of membrane into another type. Review Figure 5.18

For Discussion

1. In Chapter 47, we will see that the functioning of muscles requires calcium ions to be pumped into a subcellular compartment against a calcium concentration gradient. What types of molecules are required for this to happen?
2. Some algae have complex glassy structures in their cell walls. These structures form within the Golgi apparatus. How do these structures reach the cell wall without having to pass through a membrane?
3. Organisms that live in fresh water are almost always hypertonic to their environment. In what way is this a serious problem? How do some organisms cope with this problem?
4. Contrast nonspecific endocytosis and receptor-mediated endocytosis with respect to mechanism and to performance.



Energy, Enzymes, and Metabolism

Joseph Upton with a problem. Dr. Upton had sewn the boy's ear back on after a dog had bitten it off, but after 4 days, blood flow to and from the ear was blocked. If blood flow was not restored quickly, the reattachment would fail. To open up the blood vessels, Dr. Upton tried an old technique:

He applied 24 leeches to the wound. The leeches used their sucking mouthparts to attach to the boy's ear and drank his blood. In the process, they released a molecule called hirudin (after the scientific name of the leech, *Hirudo medicinalis*) into the boy's blood. A potent anti-clotting agent, the hirudin acted slowly but consistently over 24 hours to clear the obstructed blood vessels, and the boy's ear was saved.

The medical use of leeches to prevent blood clotting goes back thousands of years. One of the most powerful anti-clotting agents known, hirudin is a small protein of 65 amino acids that folds into a specific shape that allows it to bind tightly to thrombin, a protein present in human blood. In the absence of hirudin, thrombin has a three-dimensional structure that allows it to bind to fibrinogen (yet another blood plasma protein). When this binding occurs, a peptide bond between two of the amino acids in fibrinogen is broken, forming fibrin—the protein that forms blood clots. If hirudin binds to thrombin, thrombin cannot act on fibrinogen, and blood clots do not form.

In chemical terms, thrombin is an enzyme, or biological catalyst, for fibrin formation. The hydrolysis of peptide bonds to form fibrin would happen whether thrombin was there or not; it's just that it would happen much more slowly, certainly too slowly to have any benefit in the lifetime of the organism! Thousands of such reactions go on all the time in every organism, each reaction catalyzed by a specific protein with a particular three-dimensional structure. Taken together, these reactions make up metabolism, which is the sum total of all of the chemical conversions in a cell.

Many metabolic reactions can be classified as either (1) building up complexity in the cell, using energy to do so; or (2) breaking down complex substances into simpler ones, releasing energy in the process.

Biomedical Medicine from a Natural Source

Leeches are the source of hirudin, a molecule that prevents blood coagulation by inhibiting the action of an enzyme, thrombin, in mammalian blood.

This chapter is concerned with energy and enzymes. Without them, neither we nor any other organism would be able to function. Indeed, when an enzyme is inactivated, either by the binding of an inhibitor such as hirudin that keeps the enzyme from binding to its target, or by some error leading to an alteration in its three-dimensional structure, its function is destroyed. This can have dire consequences: What if we had hirudin in our blood all the time?

Before considering how enzymes perform their molecular wizardry, let us consider the general principles of energy in biological systems.

Energy and Energy Conversions

Physicists define energy as the capacity to do work, which occurs when a force operates on an object over a distance. In biochemistry, energy represents the capacity for change. All living things must obtain energy from the environment—no cell manufactures energy. Indeed, one of the fundamental physical laws is that energy can neither be created nor destroyed. However, energy can be transformed from one kind into another. Energy transformations are linked to the chemical transformations that occur in cells. Metabolism is the total chemical activity of a living organism; at any instant, metabolism consists of thousands of individual chemical reactions.



96 CHAPTER SIX

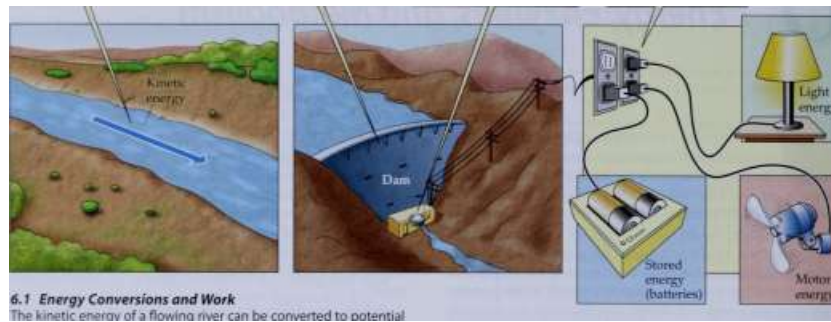
The movement of water in the river generates kinetic energy.

A dam converts the kinetic energy of a flowing river to potential energy by backing up the water and impeding its flow

\\

A generator converts the movement of water released from the dam (kinetic energy) into electric energy.

Electric energy can be transmitted, stored, and used in a variety of ways to do work.



6.1 Energy Conversions and Work

The kinetic energy of a flowing river can be converted to potential energy by a dam. Release of water from the dam converts the potential energy back into kinetic energy, which a generator can convert into electric energy.

Energy changes are related to changes in matter

Energy comes in many forms, such as chemical energy, light energy, and mechanical energy. But all forms of energy can be considered as one of two basic types:

► Kinetic energy is the energy of movement. This type of energy does work that alters the state or motion of matter. It can exist in the form of heat, light, electric, and mechanical energy, among others.

► Potential energy is the energy of state or position—that is, stored energy. It can be stored in chemical bonds, as a concentration gradient, and as electric potential, among other ways.

Water stored behind a dam has potential energy. When the water is released from the dam, some of this potential energy is converted into kinetic energy (Figure 6.1). Likewise, fatty acids, with their many C—C and C—H bonds, store chemical energy, which can be released to do biochemical work. In Chapter 5, we saw an example of the potential energy of a concentration gradient in secondary active transport, in which the gradient of one substance (Na^+) across a plasma membrane powers the transport of another (glucose) (see Figure 5.13).

In all cells of all organisms, two types of metabolic reactions occur:

► Anabolic reactions link together simple molecules to form more complex molecules. The synthesis of a protein from amino acids is anabolic. Anabolic reactions store energy in the chemical bonds that are formed.

► Catabolic reactions (catabolism) break down complex molecules into simpler ones and release stored energy.

Catabolic and anabolic reactions are often linked. The energy released in catabolic reactions is used to do biological work and drive anabolic reactions (Figure 6.2). The energy needed during anabolism to form the peptide bonds that link amino acids together into proteins comes from catabolism.

Cellular activities such as growth, motion, and active transport of ions across a membrane all require energy, and



6.2 Biological Energy Transformations

Cavorting lionesses convert chemical energy, obtained from the prey they have eaten, into a burst of kinetic energy of motion. Their prey obtained chemical energy by consuming plants. The plants trapped light energy and produced the prey's food by photosynthesis.

ENERGY, ENZYMES, AND METABOLISM 97

M

Energy transformation



The First Law of Thermodynamics. The total amount of energy before a transformation equals the total amount after a transformation. No new energy is created, and no energy is lost.

A measuring device indicates that the total energy does not change.

(b)

Energy transformation

Free energy is available to do work.

Energy before

ΔV

Usable energy after (free energy)

Unusable energy after



ΔE

This energy is not available to do work

The Second Law of Thermodynamics. Although a transformation does not change the total amount of energy within a closed system, after any transformation the amount of free energy available to do work is always less than the original amount of energy.

Closed system

.. Energy is J transformed

t

Unusable energy

Free

energy

Another statement of the Second Law is that in a closed system, with repeated energy transformations, free energy decreases and unusable energy increases—a phenomenon known as entropy.

6.3 The Laws of Thermodynamics

(a) The first law is that energy cannot be created or destroyed.

(b) The second law is that during energy transformations, free energy is lost.

none of them would proceed without a source of energy. In the discussion that follows, you will discover the physical laws that govern all energy transformations, identify the energy available to do work, and consider the direction of energy flow.

The first law: Energy is neither created nor destroyed

Energy can be converted from one form to another. For example, by striking a match, you convert potential chemical energy to light and heat. In any conversion of energy from one form to another (chemical to light, mechanical to electric), energy is neither created nor destroyed. This is the first law of thermodynamics (Figure 6.3a).

The first law applies to the universe as a whole or to any closed system within the universe. By "system" we mean any part of the universe containing specified matter and energy. A closed system is one that is not exchanging energy with its surroundings. For example, a thermos bottle does not gain or lose heat, and so the material inside it is a closed system (Figure 6Aa).

Open systems, such as living cells, exchange matter and energy with their surroundings (Figure 6Ab). Does this mean that cells disobey the first law, or that the first law does not apply to living organisms? Not at all. It means that an open system is merely one part of a larger closed system and receives energy from other parts of that larger system.

The first law tells us that in any interconversion of the forms of energy, the total energy before and after the conversion is the same. As you will see in the next two chapters, potential energy in the chemical bonds of carbohydrates and lipids can be

converted to potential energy in ATP. This energy can then be used to produce potential energy in the concentration gradients established by active transport, which can be converted to kinetic energy and used to do mechanical work, such as muscle contraction.

The second law: Not all energy can be used, and disorder tends to increase

The second law of thermodynamics states that, although energy cannot be created or destroyed, when energy is converted from one form to another, some of the energy becomes unavailable to do work (Figure 63b). In other words, no physical process or chemical reaction is 100 percent efficient, and not all the energy released can be converted to work. Some energy is lost to a form associated with disorder. The second law applies to all energy transformations, but we will focus here on chemical reactions in living systems.

not all energy can be used. In any system, the total energy includes the usable energy that can do work and the unusable energy that is lost to disorder:

total energy = usable energy + unusable energy

In biological systems, the total energy is called enthalpy (H). The usable energy that can do work is called free energy (G). Free energy is what cells require for all the chemical reactions of cell growth, cell division, and the maintenance of cell health. The unusable energy is represented by entropy (S), which is the disorder of the system, multiplied

98 CHAPTER SIX

(</>) A closed system

(b) An open system



In a closed system, no energy or matter enters or leaves.

6.4 Closed Systems and Open Systems

{a) A thermos bottle, sealed and insulated from its surroundings, is an example of a closed system, (b) A living cell, like a living individual, is an open system that must obtain energy and raw materials from its surroundings.

by the absolute temperature (T). Thus we can rewrite the word equation above more precisely as

$$H = G + TS$$

Because we are interested in usable energy, we rearrange this expression:

$$G = H - TS$$

Although we cannot measure G, H, or S absolutely, we can determine the change of each at a constant temperature. These energy changes are measured as calories (cal) or joules (J) (see Chapter 2). A change in a value is represented by the Greek letter delta (Δ), and it can be negative or positive. Therefore, the change in free energy (ΔG) of any reaction at constant temperature is defined in terms of the change in total energy (ΔH) and the change in entropy (ΔS):

$$\Delta G = \Delta H - T\Delta S$$

This equation tells us whether free energy is released or consumed by a chemical reaction. If ΔG is negative ($\Delta G < 0$), free energy is released. If ΔG is positive ($\Delta G > 0$), free energy is required (consumed). If the needed free energy is not available, the reaction does not occur.

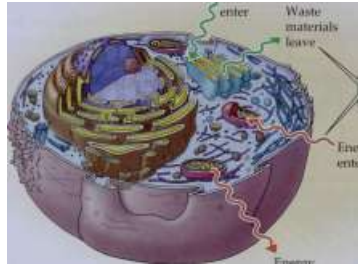
The sign and magnitude of ΔG depend on the two factors on the right of the equation: ΔH and $T\Delta S$. In a chemical reaction, ΔH is the total amount of energy added to the system ($\Delta H > 0$) or released ($\Delta H < 0$). In determining the free energy released, the sign of ΔH is obviously important. But the change in entropy must also be considered. Depending on the magnitude and sign of ΔS , the entire term, $T\Delta S$, may be negative or positive, large or small. In other words, in biological systems at constant temperature (no change

Raw

materials

enter Waste

materials leave



In an open system, energy and matter can enter and leave.

Energy enters

Energy leaves

In T), the magnitude and sign of ΔG can depend a lot on changes in entropy.

If a chemical reaction increases entropy, its products are more disordered or random. If there are more products than reactants, as in the hydrolysis of a protein to its amino acids, the products have considerable freedom to move around. The disorder and the entropy in a solution of amino acids will be large compared with that in the protein, in which peptide bonds and other forces prevent free movement. So in hydrolysis, the change in entropy (ΔS) will be positive. If there are fewer products, and they are more restrained in their movements than the reactants, ΔS will be negative. For example, a large protein linked by peptide bonds is less free in its movements than a solution of the thousands of amino acids from which it was synthesized.

Disorder tends to increase. The second law of thermodynamics also predicts that, as a result of energy conversions, disorder tends to increase in the universe or a closed system. Chemical changes, physical changes, and biological processes all tend to increase entropy and therefore tend toward disorder, or randomness (Figure 6.3b). This tendency for disorder to increase gives a directionality to physical processes and chemical reactions. It explains why some reactions proceed in one direction rather than another. The constant thermonuclear reactions in the sun will eventually result in its "running down" several billion years from now.

The second law does not say that ordered systems cannot be formed inside a large, complex closed system. In a closed system such as our solar system,* free energy can be used to create order in one part of the system. The entire

*Some energy does enter and leave the solar system—we do see the light of stars, after all—but the solar system is very nearly closed. The universe itself is a perfectly closed system (perhaps the only perfectly closed system).

ENERGY, ENZYMES, AND METABOLISM 99

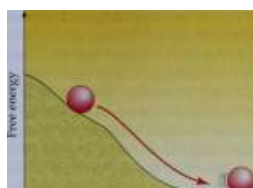
Earth, or a single cell on Earth, is an open system that receives energy from its environment. In the case of Earth, the sun provides most of that energy. In a cell, the breakdown of complex molecules in food provides the energy to create order. For example, an input of free energy results in the formation of a protein from amino acids in a solution, and an input of free energy maintains the order in your cells and in your room.

Chemical reactions release or take up energy

How are the laws of thermodynamics relevant to our understanding of the chemical reactions that occur in living things? Chemical reactions in cells are accompanied by changes in energy and changes in order. Anabolic reactions may make a single product, such as a protein (a highly ordered substance), out of many smaller amino acids (less ordered), and such reactions require or consume energy. Catabolic reactions may reduce an organized substance, such as a glucose molecule, to smaller, more randomly distributed substances, such as carbon dioxide and water, and in the process give off energy. In other words, some reactions release free energy, and others take it up.

The amount of energy released ($-\Delta G$) or taken up ($+\Delta G$) is related directly to the tendency of a reaction to run to completion (for all the reactants to form products). When a reaction goes more than halfway to completion without an input of energy, we say that it is a spontaneous reaction. Nonspontaneous reactions proceed only with the addition of free energy from the environment.

(a) Exergonic reaction (spontaneous)



► Spontaneous reactions release free energy. A reaction that releases free energy is said to be exergonic and has a negative ΔG (Figure 6.5a).

► Nonspontaneous reactions require free energy from the environment. Such reactions are said to be endergonic and have a positive ΔG (Figure 6.5b).

If a reaction runs spontaneously in one direction (from reactant A to product B, for example), then the reverse reaction (from B to A) requires a steady supply of energy to drive it:

If $A \rightarrow B$ is spontaneous and exergonic ($\Delta G < 0$),

then

$B \rightarrow A$ is nonspontaneous and endergonic ($\Delta G > 0$).

So protein hydrolysis to amino acids is spontaneous and exergonic, while protein synthesis is nonspontaneous and endergonic.

In principle, chemical reactions can run both forward and backward. For example, if compound A can be converted into compound B ($A \rightarrow B$), then B, in principle, can be converted into A ($B \rightarrow A$), although at given concentrations of A and B, only one of these directions will be favored. Think of the overall reaction as resulting from competition between forward and reverse reactions ($A \rightleftharpoons B$). Increasing the concentration of the reactants (A) speeds up the forward reaction, and increasing the concentration of the products (B) favors the reverse reaction. At some concentration of A and B, the forward and reverse reactions take place at the same rate. At this point, no further net change in the system is observable, although individual

Reactants



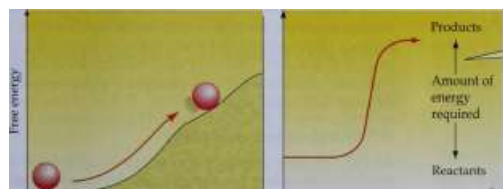
In an exergonic reaction, energy is released as the reactants form lower-energy products. ΔG is negative.

Products

(b) Endergonic reaction

(not spontaneous)

Course of reaction



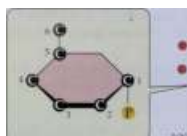
Energy must be added for an endergonic reaction, in which reactants are converted to products with a higher energy level. ΔG is positive.

Course of reaction

6.5 Exergonic and Endergonic Reactions

{a) In a spontaneous reaction, the reactants behave like a ball rolling down a hill, and energy is released—the reaction is exergonic. (b) A ball will not roll uphill spontaneously. Driving an endergonic reaction, like moving a ball uphill, requires adding free energy.

100 CHAPTER SIX



• • w # Reaction to

• • • equilibrium

• • •

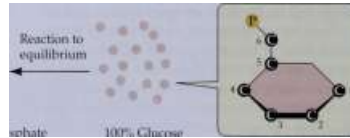
• • •

Reaction to equilibrium

100% Glucose

1-phosphate

95% Glucose 6-phosphate 5% Glucose 1-phosphate



100% Glucose 6-phosphate

In a water solution at equilibrium, both forms are present, but there is more glucose 6-phosphate than glucose 1-phosphate.

6.6 Concentration at Equilibrium

No matter what quantities of glucose 1-phosphate and glucose 6-phosphate are dissolved in water, when equilibrium is attained, there will always be 95 percent glucose 6-phosphate and 5 percent glucose 1-phosphate.

molecules are still forming and breaking apart. This balance between forward and reverse reactions is known as chemical equilibrium.

Chemical equilibrium and free energy are related

Every chemical reaction proceeds to a certain extent, but not necessarily to completion. In other words, all the reactants present are not necessarily converted to products. Each reaction has a specific equilibrium point, and that equilibrium point is related to the free energy released by the reaction under specified conditions. To understand the principle of equilibrium, consider the following example.

Every living cell contains glucose 1-phosphate, which is converted in the cell to glucose 6-phosphate. Imagine that we start out with an aqueous solution of glucose 1-phosphate that has a concentration of 0.02 M. (M stands for molar concentration; see Chapter 2.) The solution is maintained under constant environmental conditions (25°C and pH 7). As the reaction proceeds to equilibrium, the concentration of the product, glucose 6-phosphate, rises from 0 to 0.019 M, while the glucose 1-phosphate concentration falls to 0.001 M. The reaction proceeds until equilibrium is reached at these concentrations (Figure 6.6). From then on, the reverse reaction, from glucose 6-phosphate to glucose 1-phosphate, progresses at the same rate as the forward reaction.

At equilibrium, then, this reaction has a product-to-reactant ratio of 19:1 (0.019/0.001), so the forward reaction has gone 95 percent of the way to completion ("to the right," as written). Therefore, the forward reaction is a spontaneous reaction. This result is obtained every time the experiment is run under the same conditions. The reaction is described by the equation

glucose 1-phosphate \rightleftharpoons glucose 6-phosphate

The change in free energy (ΔG) for any reaction is related directly to its point of equilibrium. The further toward completion the point of equilibrium lies, the more

free energy is given off. In an exergonic reaction, such as the conversion of glucose 1-phosphate to glucose 6-phosphate, ΔG is a negative number (in this example, $\Delta G = -1.7$ kcal/mol, or -7.1 kJ/mol).

A large, positive ΔG for a reaction means that it proceeds hardly at all to the right ($A \rightarrow B$). But if the product is present, such a reaction runs backward, or "to the left" ($A \leftarrow B$), to near completion (nearly all B is converted to A). A ΔG value near zero is characteristic of a readily reversible reaction: Reactants and products have almost the same free energies.

The principles of thermodynamics we have been discussing apply to all energy exchanges in the universe—no exceptions have ever been found. Thus these principles are very powerful and useful. Next, we'll apply them to reactions in cells that involve the biological energy currency, ATP.

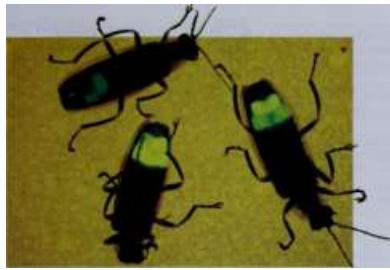
ATP: Transferring Energy in Cells

The previous chapters have mentioned adenosine triphosphate, or ATP, and its role in cells. All living cells rely on ATP for the capture, transfer, and storage of the free energy needed to do chemical work and maintain the cells (Figure 6.7). ATP operates as a kind of energy currency. That is, just as your professor teaches you, receives money for it, and then spends the money on food, some of the free energy released by certain exergonic reactions is captured in ATP, which can then release free energy to drive endergonic reactions.

ATP is produced by cells in a number of ways, which we will describe in the next two chapters, and it is used in many ways. ATP is not an unusual molecule. In fact, it has another important use in the cell: It can be converted into a building block for DNA and RNA. But there are two things about it that make it especially useful to cells: It can be hydrolyzed, and it can donate a phosphate group to many different molecules.

ATP hydrolysis releases energy

An ATP molecule consists of the nitrogenous base adenine bonded to ribose (a sugar), which is attached to a sequence of three phosphate groups (Figure 6.8). The hydrolysis of ATP yields ADP (adenosine diphosphate) and an inor-



6.7 Using ATP to Make Light

Fireflies convert the energy of chemical bonds in ATP into light energy, emitting rhythmic flashes that signal the insect's readiness to mate. Very little of the energy in this conversion is lost as heat.

ganic phosphate ion (abbreviated P_i in short for HPO_4^{2-}), as well as free energy:



Two important properties of this reaction are:

- It is exergonic, releasing free energy. The change in free energy (ΔG) is about -12 kcal/mol (-50 kJ/mol) at the temperature, pH, and substrate concentrations typical of living cells.*
- The equilibrium for this reaction is far to the right—that is, toward ADP production. At equilibrium in the cell, there is 10 million times as much ADP as ATP.

What characteristics of ATP account for the free energy released by the hydrolysis of its phosphates? Consider how phosphates are added to adenosine monophosphate—AMP—to make ADP. The phosphate on AMP is negatively charged; so is the free phosphate to be added. It takes a lot of free energy to overcome the tendencies of these phosphates to repel each other. The same thing happens with the addition of the third phosphate to ADP (adenosine diphosphate) to make ATP. Once again, the two negatively charged molecules repel each other unless a lot of energy is added. An analogy is the springs that suspend a car: To compress the springs, you need to lean on the car (input of energy), and the car bounces up (the energy is released) when the springs extend. Likewise, the energy required to make ATP is released when it is hydrolyzed.

ATP couples exergonic and endergonic reactions

As we have just seen, the hydrolysis of ATP is exergonic and yields ADP, P_i , and free energy. The reverse reaction, the formation of ATP from ADP and P_i , is endergonic and

"The "standard" ΔG for ATP hydrolysis is -7.3 kcal/mol or -30 kJ/mol , but that value is valid only at pH 7 and with ATP, ADP, and phosphate present at concentrations of 1 M —concentrations that differ greatly from those found in cells.

ATP

(space-filling

model)



ATP (structural formula) Adenine NH- >

N

<?

H—C

^

C

c

N

^

C-H

N'

N

Phosphate groups

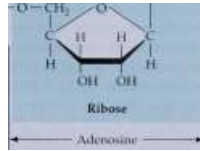
O

O-

o-

O — CH-

O"



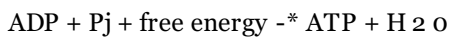
— AMP (Adenosine monophosphate) ADP (Adenosine diphosphate)

ATP (Adenosine triphosphate)

6.8 Structures of ATP, ADP, and AMP

ATP is richer in energy than its relatives ADP and AMP. The hydrolysis of ATP releases this energy.

consumes as much free energy as is released by the breakdown of ATP:



Many different enzyme-catalyzed exergonic reactions in the cell can provide the energy to convert ADP to ATP. In eukaryotes, the most important of these reactions is cellular respiration, in which energy is released from fuel molecules and trapped in ATP. The formation and hydrolysis of ATP constitute what might be called an "energy-coupling cycle," in which ATP shuttles energy from exergonic reactions to endergonic reactions.

How does this ATP cycle trap and release energy? An exergonic reaction is coupled to the endergonic reaction that forms ATP from ADP and P_i (Figure 6.9). Coupling of exergonic and endergonic reactions is very common in biochemistry. When it forms, ATP captures free energy. ATP then diffuses to another site in the cell, where its hydrolysis releases free energy to drive an endergonic reaction.

A specific example of this energy-coupling cycle is shown in Figure 6.10. The formation of the amino acid glutamine has a positive ΔG (is endergonic, nonspontaneous) and will not proceed without the input of free energy from ATP hydrolysis, which has a negative ΔG (exergonic, spontaneous). The total ΔG for the coupled reactions is negative (the two ΔG 's are added together). Hence the reactions proceed spontaneously when they are coupled, and glutamine is synthesized.

102 CHAPTER SIX



Energy

energy

Hydrolysis of ATP to ADP and P_i releases energy.

Endergonic active transport: against a concentration gradient

Endergonic cell movements

Anabolism:

endergonic synthesis of macromolecules: proteins, polysaccharides, lipids

O

6.9 Formation and Use of ATP

Exergonic cellular processes release the energy needed to create ATP from ADP. The energy released from the conversion of ATP back to ADP can be used to fuel endergonic processes.

Actually, the overall reaction proceeds in two steps and involves a phosphorylated intermediate that is common to both reactions:

- In the first reaction, ATP transfers a phosphate group to glutamate (glutamic acid), producing the higher-energy product glutamyl phosphate (the phosphorylated intermediate).
- In the second reaction, the hydrolysis of glutamyl phosphate provides sufficient free energy to drive the reaction with an ammonium ion (NH_4^+) to form glutamine.

An active cell requires millions of molecules of ATP per second to drive its biochemical machinery. An ATP molecule is consumed within a minute following its formation, on average. At rest, an average person hydrolyzes and produces about 40 kg of ATP per day—as much as some people weigh! This means that each ATP molecule undergoes about 10,000 cycles of synthesis and hydrolysis every day.

Enzymes: Biological Catalysts

When we know the change in free energy (ΔG) of a reaction, we know where the equilibrium point of the reaction lies: The more negative ΔG is, the further the reaction proceeds toward completion. However, ΔG tells us nothing about the rate of a reaction—the speed at which it moves toward equilibrium. As we will see, some exergonic reac-

This reaction is exergonic and releases energy

Exergonic reaction

ATP

+ H_2O

Endergonic reaction

/

-C + NH_4^+

Energy

ADP +

$\Delta G = -7.3 \text{ kcal/mol}$

O

tions are very rapid; others are slower. Living cells cope with this variability by using biological catalysts to increase the rates of almost all chemical reactions.

A catalyst is any substance that speeds up a chemical reaction without itself being used up. A catalyst does not cause a reaction to take place that would not take place eventually without it, but merely speeds up the rates of both forward and backward reactions, allowing equilibrium to be approached faster. Most biological catalysts are proteins called enzymes. Although we will focus here on proteins, you should know that certain RNA molecules, called ribozymes, are also catalytic. Indeed, in the evolution of life, catalytic RNA may have preceded catalytic proteins (see Chapter 25).

In the discussion that follows, we will identify the energy barrier that controls the rate of reactions. Then we'll focus on the role of enzymes: how they interact with reactants, how they lower the activation energy barrier, and how they permit reactions to proceed faster. After exploring the nature and significance of enzyme specificity, we'll look at how enzymes contribute to the coupling of reactions.

For a reaction to proceed, an energy barrier must be overcome

An exergonic reaction may release a great deal of free energy, but the reaction may take place very slowly. Some reactions are slow because there is an energy barrier between reactants and products. Think about a butane lighter. The burning of the butane gas ($\text{C}_4\text{H}_{10} + 13\text{O}_2 \rightarrow 4\text{CO}_2 + 10\text{H}_2\text{O}$) is obviously exergonic—heat and light are released. Once started, the reaction goes to completion: All of the butane reacts with oxygen to form carbon dioxide and water vapor.

The ΔG indicates an exergonic reaction.

/

The $+\Delta G$ indicates an endergonic reaction.

>

\

— C

O"

NH,

AG = +3.4 kcal/mol

AG = -3.9 kcal/mol OVERALL

~Y~

Glutamate (part)

Y Glutamine

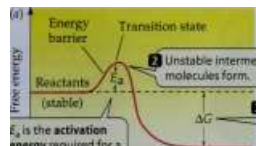
This reaction is endergonic and will not occur without an input of energy.

The coupled reaction has an overall - AG, indicating an exergonic reaction and so is spontaneous.

6.10 Coupling ATP Hydrolysis to an Endergonic Reaction

The synthesis of the amino acid glutamine from glutamate and an ammonium ion is endergonic and must be coupled with the exergonic hydrolysis of ATP.

Transition state



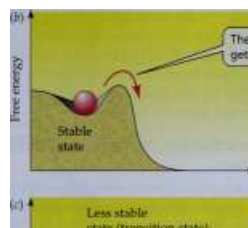
stable intermediate molecules form.

Q E a is the activation energy required for a reaction to begin.

Q AG for the reaction is not affected by F a .

Products

Course of reaction



The ball needs a push (E a) to get it out of the depression.

i-

c

01

Less stable

state (transition state)



A ball that has received an input of activation energy can roll downhill spontaneously, releasing free energy.

6.7 7 Activation Energy initiates Reactions

(a) In any chemical reaction, an initial stable state must become less stable before change is possible. (b,c) A ball on a hillside provides a physical analogy to the biochemical principle graphed in (a).

Because burning butane liberates so much energy, you might expect this reaction to proceed rapidly whenever butane is exposed to oxygen. But this does not happen. Simply mixing butane with air produces no reaction. Butane will start burning

only if a spark—an input of energy—is provided. (In the butane lighter, this is supplied by friction.) The need for this spark to start the reaction shows that there is an energy barrier between the reactants and the products.

In general, exergonic reactions proceed only after they are pushed over the energy barrier by a small amount of added energy. The energy barrier thus represents the amount of energy needed to start the reaction. This amount is called the activation energy, and is symbolized E_a (Figure 6.11). Recall the ball rolling down the hill in Figure 6.5. The ball has a lot of potential energy at the top of the hill. However, if the ball is stuck in a small depression, it won't roll down the hill, even though that action is exergonic (Figure 6.11b). To start the ball rolling, a small amount of energy (activation energy) is needed to get the ball out of the depression (Figure 6.11c).

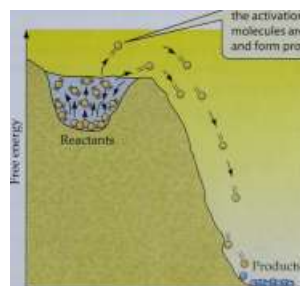
In a chemical reaction, the activation energy is the energy needed to change the reactants into unstable molecular forms called transition-state species. Transition-state species have higher free energies than either the reactants or the products. Their bonds may be stretched and hence unstable. Although the amount of activation energy needed for different reactions varies, it is often small compared with the change in free energy of the reaction. The activation energy that starts a reaction is recovered during the ensuing "downhill" phase of the reaction, so it is not a part of the net free energy change, ΔG (see Figure 6.11f).

Where does the activation energy come from? In any collection of reactants at room or body temperature, molecules are moving around and could use their kinetic energy of motion to overcome the energy barrier, enter the transition state, and react (Figure 6.12). However, at normal temperatures, only a few molecules have enough energy to do this; most have insufficient kinetic energy for activation. If the system were heated, all the reactant molecules would move faster and have more kinetic energy. Since more of them would have energy exceeding the required activation energy, the reaction would speed up.

However, adding enough heat to increase the average kinetic energy of the molecules is not an effective option for living systems. Such a general, nonspecific approach would accelerate all the reactions, including destructive ones, such as the denaturation of proteins (see Chapter 3). Another, biologically more effective way to speed up a reaction is to lower the activation energy barrier. In living cells, enzymes accomplish this task.

Enzymes bind specific reactant molecules

All types of catalysts speed chemical reactions. Most non-biological catalysts are nonspecific and work on a variety



The kinetic energy of some molecules equals or exceeds the activation energy. These molecules are able to react and form products.

Course of reaction

6.72 Over the Energy Barrier

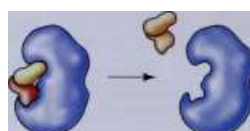
Some molecules have enough kinetic energy to surmount the energy barrier and react, forming products. At the temperatures of most organisms, this is a small proportion of the molecules.

104 CHAPTER SIX

Product

Substrates match the active site...

...but nonsubstrate does not.



The breakdown of the enzyme-substrate complex yields the product. The enzyme is now available to catalyze another reaction.

Enzyme

6.73 Enzyme and Substrate

An enzyme is a protein catalyst with an active site capable of binding one or more substrate molecules. The enzyme-substrate complex yields product and free enzyme.

of reactants. For example, powdered platinum catalyzes virtually any reaction in which molecular hydrogen (H_2) is a reactant. In contrast, most biological catalysts are proteins called enzymes, and they are highly specific. An enzyme usually recognizes and binds to only one or a few closely related reactants, and it catalyzes only a single chemical reaction.

In an enzyme-catalyzed reaction, the reactants are called substrates. Substrate molecules bind to a particular site on the enzyme surface, called the active site, where catalysis takes place (Figure 6.13). The specificity of an enzyme results from the exact three-dimensional shape and structure of its active site, into which only a narrow range of substrates fit. Other molecules—with different shapes, different functional groups, and different properties—cannot properly fit and bind to the active site.

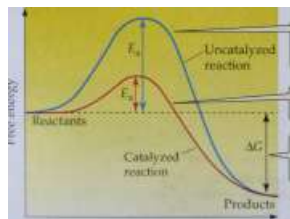
The names of enzymes reflect the specificity of their functions. For example, the enzyme RNA polymerase will catalyze the formation of RNA but not DNA, and the enzyme hexokinase accelerates the phosphorylation of hex-ose sugars, but not pentose sugars. Most, but not all, names of enzymes end in the suffix "-ase."

The binding of a substrate to the active site produces an enzyme-substrate complex held together by one or more means, such as hydrogen bonding, ionic attraction, or co-valent bonding. The enzyme-substrate complex gives rise to product and free enzyme:



Enzyme-substrate complex

Enzyme



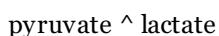
An uncatalyzed reaction has a greater activation energy than does a catalyzed reaction.

where E is the enzyme, S is the substrate, P is the product, and ES is the enzyme-substrate complex. The free enzyme (E) is in the same chemical form at the end of the reaction as at the beginning. While bound to the substrate, it may change chemically, but by the end of the reaction it has been restored to its initial form.

Enzymes lower the activation energy barrier but do not affect equilibrium

The formation of an enzyme-substrate complex results in a lower activation energy than the transition-state species of the corresponding uncatalyzed reaction (Figure 6.14). Thus the enzyme lowers the energy barrier for the reaction—it offers the reaction an easier path. When an enzyme lowers the activation energy barrier, both the forward and the reverse reactions speed up, so the enzyme-catalyzed overall reaction proceeds toward equilibrium more rapidly than the uncatalyzed reaction. The final equilibrium (and ΔG) is the same with or without the enzyme.

The enzyme lactate dehydrogenase, for example, catalyzes the highly reversible reaction



We will study this reaction in the next chapter. But for now, what is the substrate for this reaction? The answer is, either pyruvate or lactate. When we exercise vigorously, the reaction proceeds to the right because there is a lot of pyruvate around. Lactate builds up in our muscles (and we get cramps). Lactate moves into the blood, and the blood system takes it from the muscles to the liver, where lactate dehydrogenase catalyzes its conversion back to pyruvate. In the meantime, the lactate concentration in our muscles is now lower, so lactate dehydrogenase in the muscles catalyzes the conversion of pyruvate to lactate. This is an excellent example of how concentrations of substrates affect the net direction of a reversible reaction.

Adding an enzyme to a reaction does not change the difference in free energy (ΔG) be-

A catalyzed reaction has a lower activation energy.

There is no difference in free energy between catalyzed and uncatalyzed reactions.

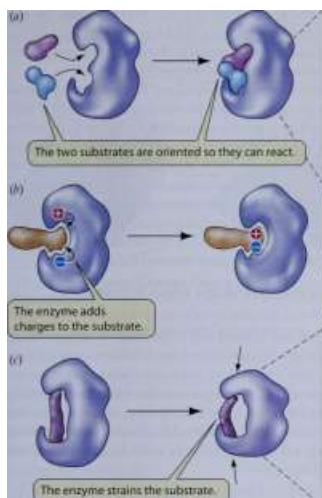
©.

Course of reaction

6.14 Enzymes Lower the Activation Energy Barrier

Although the activation energy is lower in an enzyme-catalyzed reaction than in an uncatalyzed reaction, the energy released is the same with or without catalysis. In other words, E_a is lower, but ΔG is unchanged.

ENERGY, ENZYMES, AND METABOLISM 105



Wh

Citrate synthase

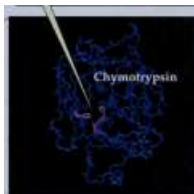
/

Two substrates are bound at the active site of the enzyme citrate synthase.

Two amino acids at the active site of chymotrypsin participate in altering the substrate.

the activation energy is used to make the substrates collide with the right atoms for bond formation next to each other.

When proteins are synthesized, for example, a peptide bond is formed between the carboxyl group of one amino acid and the amino group of the next (see Figure 3.4). For two amino acids to collide and form a peptide bond, these two chemical groups must be the sites of collision. When the active site of an enzyme binds to one amino acid, it is held in the right orientation to react with a second amino acid substrate when it binds to the enzyme.



6.75 Life at the Active Site

Enzymes have several ways of causing their substrates to enter the transition state, (a) Orientation, (b) Chemical charge, (c) Physical strain.

tween the reactants and the products (see Figure 6.14). It does change the activation energy and, consequently, the rate of reaction. If 600 molecules of a protein with arginine as its terminal amino acid just sit in solution, thermodynamically the proteins tend toward disorder and the terminal peptide bonds break, releasing the arginines (ΔS increases). After 7 years, about half (300) of the proteins will have undergone this spontaneous reaction. With the enzyme carboxypeptidase A catalyzing the reaction, the 300 arginines are released in half a second!

What are the chemical events at active sites of enzymes?

How does an enzyme speed up the rate of a reaction? Like any substance that binds to a protein, a substrate interacts with the active site of an enzyme by shape and by chemical interactions. The chemical interactions contribute directly to the breaking of old bonds and the formation of new ones (Figure 6.15). In catalyzing a reaction, an enzyme may use one or more of the following mechanisms.

enzymes orient substrates. While free in solution, substrates are rotating and tumbling around and may not have the proper orientation to interact when they collide. Part of

ENZYMES ADD CHARGES TO SUBSTRATES.

The side chains (R groups) of an enzyme's amino acids may be direct participants in making its substrates more chemically reactive. For example, in acid-base catalysis, the acidic or basic side chains of the amino acids forming the active site may

transfer H⁺ to or from the substrate, destabilizing a covalent bond in the substrate and permitting it to break. In covalent catalysis, a functional group in a side chain forms a temporary covalent bond with a portion of the substrate. In metal ion catalysis, metal ions such as copper, zinc, iron, and manganese, which are firmly bound to side chains of the protein, can lose or gain electrons without altering the bonds that hold them to the protein. This ability makes them important participants in oxidation-reduction reactions, which involve loss or gain of electrons.

ENZYMES INDUCE STRAIN IN THE SUBSTRATE. Once a Substrate

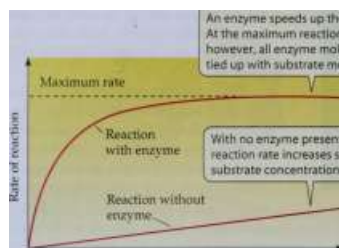
has bound to the active site, the enzyme can cause bonds in the substrate to stretch, putting it in an unstable transition state. For example, the carbohydrate substrate for the enzyme lysozyme (see Figure 6.17) enters the active site in a flat-ringed "chair" shape, but the active site quickly causes it to flatten out into a "sofa." The resulting stretching of its bonds causes them to be less stable and more reactive to the other substrate, water.

Substrate concentration affects reaction rate

For a reaction of the type $A \rightarrow B$, the rate of the uncatalyzed reaction is directly proportional to the concentration of A (Figure 6.16). The higher the concentration of substrate, the more collisions, and the more reactions per unit of time (higher rate). Addition of the appropriate enzyme speeds up the reaction, of course, but it also changes the shape of the plot of rate versus substrate concentration. At first, the rate of the enzyme-catalyzed reaction increases as the substrate concentration increases, but then it levels off.

106 CHAPTER SIX

An enzyme speeds up the reaction. At the maximum reaction rate, however, all enzyme molecules are tied up with substrate molecules.



With no enzyme present, the reaction rate increases steadily as substrate concentration increases.

Concentration of substrate

6.76 Enzymes Speed Up Reaction Rates

Because there is usually less enzyme than substrate present, the reaction rate levels off when the enzyme becomes saturated.

When further increases in the substrate concentration do not significantly increase the reaction rate, the maximum rate is attained.

Since the concentration of an enzyme is usually much lower than that of its substrate, what we are seeing is a saturation phenomenon like the one that occurs in facilitated diffusion (see Chapter 5). When all the enzyme molecules are bound to substrate molecules, the enzyme is working as fast as it can—at its maximum rate. Nothing is gained by adding more substrate, because no free enzyme molecules are left to act as catalysts.

The maximum rate of an enzyme reaction can be used to measure how efficient the enzyme can be—that is, how many molecules of substrate are converted to product per unit of time when there is an excess of substrate present. This turnover number ranges from 1 molecule every 2 seconds for lysozyme (see Figure 6.17) to an amazing 40 million molecules per second for the liver enzyme catalase.

Molecular Structure Determines Enzyme Function

Most enzymes are much larger than their substrates. An enzyme is typically a protein with hundreds of amino acids, and its substrate is generally not a macromolecule at all, but a small-molecule metabolite. The active site of the enzyme is usually quite small, not more than 6-12 amino acids. Two questions arise from this observation:

- What is the nature of the active site that allows it to recognize and bind the substrate?
- What is the role of the rest of the huge protein?

The active site is specific to the substrate

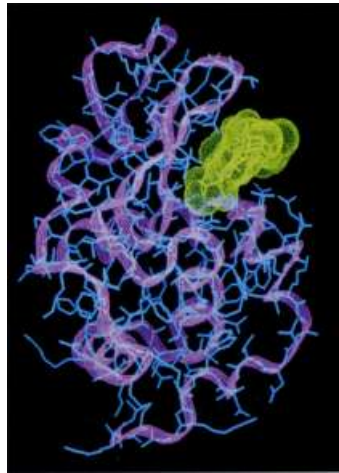
The remarkable ability of an enzyme to select exactly the right substrate depends on a precise interlocking of molecular shapes and interactions of chemical groups at the binding site. The binding of the substrate to the active site

depends on the same kinds of forces that maintain the tertiary structure of the enzyme: hydrogen bonds, the attraction and repulsion of electrically charged groups, and hydrophobic interactions (see Chapter 3).

In 1894, the German chemist Emil Fischer compared the fit between an enzyme and its substrate to that of a lock and key. Fischer's model persisted for more than half a century with only indirect evidence to support it. The first direct evidence came in 1965, when David Phillips and his colleagues at the Royal Institution in London succeeded in crystallizing the enzyme lysozyme and determined its tertiary structure using the techniques of X-ray crystallography (described in Chapter 11). They observed a pocket in lysozyme that neatly fits its substrate (Figure 6.17).

An enzyme changes shape when it binds a substrate

Fischer's idea has turned out to be largely correct, with one modification. Studies on enzyme inhibitors were done to see if molecules similar to a substrate could fit into an active site on an enzyme and prevent the real substrate from binding. The first such experiments were successful in that the mimic "substrates" did bind to the enzyme, but they did not react. Likewise, a false key can fit into a lock, but the lock will not open.



6.7 Tertiary Structure of Lysozyme

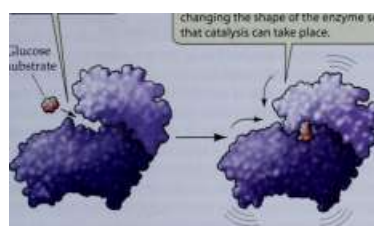
Lysozyme is an enzyme that protects the animals that produce it by destroying invading bacteria. To destroy the bacteria, it cleaves certain polysaccharide chains in their cell walls. Lysozyme is found in tears and other bodily secretions, and it is particularly abundant in the whites of bird eggs. The active site of lysozyme appears as an indentation filled with the substrate (shown in yellow).

t

Hexokinase with an empty active site

Glucose substrate

When the substrate binds to the active site, the two side chains move together, changing the shape of the enzyme so that catalysis can take place.



6.18 Some Enzymes Change Shape When Substrate Binds to Them

Shape changes result in an induced fit between enzyme and substrate, improving the catalytic ability of the enzyme.

Then, remarkably, some enzyme inhibitors were found that were considerably larger than the real substrate, yet were effective. How could a key twice as large as the real one fit into the lock? It is here that the Fischer lock and key analogy breaks down.

The answer is that enzymes are flexible, and their active sites can change (expand) to fit substrates. When the substrate binds, the enzyme changes shape, exposing the parts of itself that react with the substrate. This change in enzyme shape caused by substrate binding is called induced fit.

Induced fit can be observed in the enzyme hexokinase (Figure 6.18) when it is studied with and without one of its substrates, glucose (its other substrate is ATP). It catalyzes the reaction



A Few Examples of Nonprotein Molecular "Partners" of Enzymes

TYPE OF MOLECULE

ROLE IN CATALYZED REACTIONS

Induced fit brings reactive side chains from the enzyme's active site into alignment with the substrates, facilitating the catalytic mechanisms described earlier (see Figure 6.15).

Equally important, the folding of hexokinase to fit around the glucose substrate excludes water from the active site. This is essential, because the two molecules binding to the active site are glucose and ATP. If water were present, ATP would be rapidly hydrolyzed to ADP and phosphate. But since water is absent, the transfer of a phosphate from ATP to glucose is favored.

Induced fit at least partly explains why enzymes are so large. The rest of the molecule may have two roles:

- ▶ It provides a framework so that the amino acids of the active site are properly positioned in relation to the substrate.
- ▶ It participates in the small but significant changes in protein shape and structure that result in induced fit.

To operate, some enzymes require added molecules

Whether they consist of a single folded polypeptide chain or several subunits, many enzymes require other, nonprotein molecules in order to function (Table 6.1).

- ▶ Cofactors are inorganic ions such as copper, zinc, and iron that bind temporarily to certain enzymes and are essential to their function.
- ▶ Coenzymes are carbon-containing molecules that are required for the action of one or more enzymes. Coenzymes are usually relatively small compared with the enzyme to which they temporarily bind (Figure 6.19).

6.79 An Enzyme with a Coenzyme

Some enzymes require coenzymes in order to function. This illustration shows the relative sizes of the four subunits (red, orange, green, and purple) of the enzyme glyceraldehyde-3-phosphate dehydrogenase and its coenzyme, NAD (white).

108 CHAPTER SIX

- ▶ Prosthetic groups are permanently bound to their enzymes. They include the heme groups that are attached to the oxygen-carrying protein hemoglobin (see Figure 3.7).

Because coenzymes are not permanently bound to the enzyme, they must react with it as a substrate does. For the catalyzed reaction to proceed, coenzymes must collide with the enzyme and bind to its active site just as the substrate must. A coenzyme can be considered a substrate because it changes chemically during the reaction and then separates from the enzyme to participate in other reactions. Coenzymes move from enzyme molecule to enzyme molecule, adding or removing chemical groups from the substrate.

ATP and ADP can be considered coenzymes, because they are necessary for some reactions, are changed by reactions, and bind to and detach from the enzyme. In the next chapter, we will encounter coenzymes that function in energy processing by accepting or donating electrons or hydrogen atoms. In animals, some coenzymes are produced from vitamins that must be obtained from food—they cannot be synthesized by the body.

Metabolism and the Regulation of Enzymes

All organisms need to maintain stable internal conditions, or homeostasis. Thus we and all other organisms must regulate our metabolisms. The regulation of the rates at which our thousands of different enzymes operate contributes to metabolic homeostasis.

In the remainder of this chapter, we will investigate the role of enzymes in organizing and regulating metabolism. In living cells, the activity of enzymes can be inhibited in various ways, so the presence of an enzyme does not necessarily ensure that it is functioning. There are mechanisms to alter the rate at which some enzymes catalyze reactions, making enzymes the target points at which entire sequences of chemical reactions can be regulated. Finally, we examine how the environment—namely, temperature and pH—affects enzyme activity.

Metabolism is organized into pathways

An organism's metabolism is the totality of the biochemical reactions that take place within it. Metabolism transforms raw materials and stored potential energy into forms that can be used by living cells. Metabolism consists of sequences of enzyme-catalyzed chemical reactions called pathways. In these sequences, the product of one reaction is the substrate for the next:

cell's (and the organism's) needs. So a cell must regulate all its metabolic pathways constantly.

Enzyme activity is subject to regulation

Various inhibitors can bind to enzymes, slowing down the rates of enzyme-catalyzed reactions. Some inhibitors occur naturally in cells; others are artificial. Naturally occurring inhibitors regulate metabolism; artificial ones can be used to treat disease, to kill pests, or in the laboratory to study how enzymes work. Some inhibitors irreversibly inhibit the enzyme by permanently binding to it. Others have reversible effects; that is, they can become unbound from the enzyme. The removal of a natural reversible inhibitor increases an enzyme's rate of catalysis.

irreversible inhibition. Some inhibitors irreversibly covalently bond to certain side chains at active sites of enzymes, thereby inactivating the enzymes by destroying their capacity to interact with the normal substrate. Such inhibitors are generally not natural products. A compound called DIPF (diisopropylphosphorofluoridate), for example, reacts with a hydroxyl group of the amino acid serine at an enzyme's active site, preventing the use of this side chain in catalytic reactions (Figure 6.20). DIPF is an irreversible inhibitor for the protein-digesting enzyme trypsin and for many other enzymes whose active sites contain serine. Another DIPF-inhibited enzyme is acetylcholinesterase, which is essential for the orderly propagation of impulses from one nerve cell to another. Because of their effect on acetylcholinesterase, DIPF and other similar compounds are classified as nerve gases. One of them, Sarin, was used in an attack on the Tokyo subway in 1995, resulting in a dozen deaths and hundreds hospitalized. The widely used insecticide malathion is a derivative of DIPF that inhibits only insect acetylcholinesterase and not the mammalian enzyme.

reversible inhibition. Not all inhibition is irreversible. Some inhibitors are similar enough to a particular enzyme's natural substrate to bind to the active site, yet dif-

Active site of trypsin

enzyme

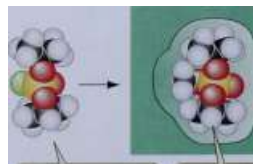
B

enzyme

*c

enzyme

D



+

Hydrogen fluoride

Some metabolic pathways are anabolic, and synthesize the important chemical building blocks from which macromolecules are built. Others are catabolic, breaking down molecules for usable free energy. The balance among these anabolic and catabolic pathways may change depending on the

The hydroxyl group is on the side chain of serine in the active site.

DIPF, an irreversible inhibitor, reacts with the hydroxyl group of serine.

Permanent attachment of DIPF to the active site prevents substrate from entering, thus disabling the enzyme.

6.20 Irreversible Inhibition

DIPF forms a stable covalent bond with the side chain of the amino acid serine at the active site of the enzyme trypsin.

ferent enough that the enzyme catalyzes no chemical reaction. While such a molecule is bound to the enzyme, the natural substrate cannot enter the active site; thus the inhibitor effectively wastes the enzyme's time, preventing its catalytic action. Such molecules are called competitive inhibitors because they compete with the natural substrate for the active site (Figure 6.21a). In these cases, the inhibition is reversible. When the concentration of the competitive inhibitor is reduced, it detaches from the active site, and the enzyme is again active.

The enzyme succinate dehydrogenase is subject to competitive inhibition. This enzyme, found in all mitochondria, catalyzes the conversion of the compound succinate to another compound, fumarate. The compound oxaloacetate is similar to succinate and can act as a competitive inhibitor of succinate dehydrogenase by binding to its active

6.27 Reversible Inhibition

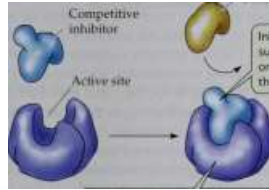
(a) In competitive inhibition, an inhibitor binds temporarily to the active site. Succinate dehydrogenase, for example, is subject to competitive inhibition by oxaloacetate. (f) A noncompetitive inhibitor binds temporarily to the enzyme at a site away from the active site, but still prevents the enzyme from functioning.



(a) Competitive inhibition

Competitive inhibitor

Substrate



Inhibitor and substrate "compete," only one can bind to the active site.

Enzyme

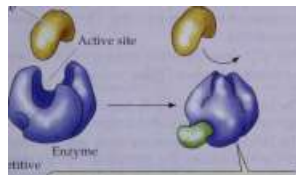
The enzyme's function is disabled as long as the inhibitor remains bound. However, should the inhibitor become unbound, a substrate molecule can bind to the active site.

(b) Noncompetitive inhibition

Substrate

c?

Noncompetitive inhibitor



Enzyme

An inhibitor may bind to a site away from the active site, changing the enzyme's shape so that the substrate no longer fits.

site. However, having bound to oxaloacetate, the enzyme can do nothing more with it—no reaction occurs. An enzyme molecule cannot bind a succinate molecule until the oxaloacetate molecule has moved out of the active site.

Some inhibitors that do not react with the active site are called noncompetitive inhibitors. Noncompetitive inhibitors bind to the enzyme at a site distinct from the active site. Their binding can cause a conformational change in the enzyme that alters the active site (Figure 6.21b). In this case, the active site may still bind substrate molecules, but the rate of product formation may be reduced. Noncompetitive inhibitors, like competitive inhibitors, can become unbound, so their effects are reversible.

Allosteric enzymes have interacting subunits

Many important enzymes have a quaternary structure consisting of two or more polypeptide subunits, each with a molecular weight in the tens of thousands (see Chapter 3). These subunits are bound together by various weak bonds that permit changes in the shape of one subunit to influence the shape and properties of the others. Multisubunit enzymes that undergo such changes in shape and function

Competitive inhibition of succinate dehydrogenase



+ AH,

Succinate (substrate)

«Vj*

Fumarate

Catalyzed by

succinate dehydrogenase

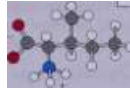
Oxaloacetate (competitive inhibitor)

Succinate dehydrogenase is subject to competitive inhibition by oxaloacetate, which resembles succinate enough to bind to the active site but cannot react.

Noncompetitive inhibition of threonine dehydratase

1 threonine

(substrate)



Catalyzed by

threonine dehydratase

$>V^{\wedge}$

α -Ketobutvrate

Isoleucine

(noncompetitive inhibitor)

Threonine dehydratase is subject to noncompetitive inhibition by isoleucine, which alters the enzyme by binding away from the active site.

110 CHAPTER SIX

(<.) Single-subunit enzyme

(/») Allosteric (multiple-subunit) enzyme



Concentration of substrate

6.22 Allostery and Reaction Rate

How the rate of an enzyme-catalyzed reaction changes with increasing substrate concentration depends on whether the enzyme consists of one or more than one polypeptide subunit.

are called allosteric enzymes (alio-, "different"; -steric, "shape"). (Note that not all allosteric enzymes have multiple subunits; there are some single-chain enzymes that are al-losterically regulated.)

The activity of allosteric enzymes is controlled by molecules called effectors, which may have no structural similarity either to the reactants or to the products of the reaction being catalyzed. Effectors bind to an allosteric site that is separate from the active site, changing the structure of the enzyme and thus its activity. Their binding may enhance or diminish reactions at the active site; thus, effectors may be activators or inhibitors.

Allosteric enzymes and nonallosteric enzymes differ greatly in their reaction rates when the substrate concentration is low. Graphs of reaction rate plotted against substrate concentration show this relationship. For an enzyme with a single subunit, the plot looks like that in Figure 6.22a. The reaction rate first increases very sharply with increasing substrate concentration, then tapers off to a constant maximum rate as the supply of enzyme becomes saturated with substrate. The plot for many allosteric enzymes is radically different, having a sigmoidal (S-shaped) appearance (Figure 6.22b). The increase in rate with increasing substrate concentration is slight at low substrate concentrations, but within a certain range the reaction rate is extremely sensitive to relatively small changes in substrate concentration. Because of this sensitivity, allosteric enzymes are important in regulating entire pathways and activities of a cell. We can understand this behavior in terms of interactions between the different kinds of subunits that make up an allosteric enzyme, which we'll examine next.

CATALYTIC AND REGULATORY SUBUNITS INTERACT AND COOPERATE.

An allosteric enzyme not only usually has more than one subunit, it usually has more than one type of subunit:

- ▶ A catalytic subunit has an active site that binds the enzyme's substrate.
- ▶ A regulatory subunit has one or more allosteric sites that bind specific effector molecules.

Binding of either a substrate or an effector affects the structure of the enzyme as a whole.

An allosteric enzyme can exist in two forms. The active form has catalytic activity, whereas the inactive form lacks activity. When the enzyme is in its active form, the active sites on the catalytic subunits can accept substrate. When the enzyme is in its inactive form, the allosteric sites on the regulatory subunits can accept inhibitor.

An allosteric enzyme usually consists of two or more catalytic subunits and one or more regulatory subunits. The existence of two or more linked catalytic subunits allows for cooperativity, in which one subunit's activity influences the activity of its neighbors.

When a molecule of substrate binds to the active site of one catalytic subunit of an allosteric enzyme, it causes a change in the other catalytic subunits, making it easier for substrate to bind to them (Figure 6.23). Conversely, when an allosteric inhibitor binds to the allosteric site of a regulatory subunit, it causes a different change in the catalytic subunits, making it harder for substrate to bind to them. This cooperativity between subunits makes an enzyme exquisitely sensitive to its molecular environment. The binding of just one substrate molecule makes it easier for further substrate molecules to react.

allosteric effects regulate metabolism. Metabolic pathways typically involve a starting material, various intermediates, and a product, which is used for some purpose by the cell. In each pathway, there are a number of reactions, each forming an intermediate, and each catalyzed by a different enzyme. The first step in a pathway is called the commitment step, meaning that once this enzyme-catalyzed conversion occurs, the "ball is rolling," and the other conversions happen in sequence, leading to the final product. But what if the cell has no need for that product—for example, if it takes it up from its environment in adequate amounts? It would be energetically wasteful for the cell to continue making something it does not need.

One way that cells solve this problem is to shut down the metabolic pathway by having the final product allosterically inhibit the enzyme that catalyzes the commitment step (Figure 6.24). This mechanism is known as end-product inhibition. When the end product is present in a high concentration, some of it binds to an allosteric site on the commitment step enzyme, thereby causing it to become inactive.

We will describe other examples of allosteric interactions in later chapters. These include:

- ▶ The binding of oxygen to hemoglobin, which shows a sigmoid relationship and cooperativity (see Chapter 49).
- ▶ The binding of a hormone to its cellular receptor protein, which causes the protein to change shape and provides the signal to initiate reactions within the cell (see Chapter 15).

► The binding of an inducer to a protein that regulates DNA expression (see Chapter 12).

The hypothetical enzyme shown here has four subunits, two catalytic subunits (blue) and the other two regulatory subunits (pink).

Conformational change

The enzyme switches back and forth between the two forms. They are in equilibrium.

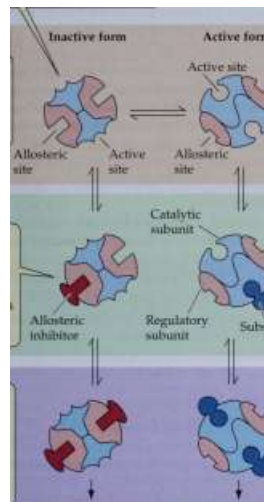
Allosteric regulation

Active form

When the enzyme is in its inactive form, the allosteric sites on the regulatory subunits can accept inhibitor.

Cooperativity

Once a site is filled with a substrate or an inhibitor, binding at a second site of the same type is favored.



When the enzyme is in its active form, the active sites on the catalytic subunits can accept substrate

Substrate

ENERGY, ENZYMES, AND METABOLISM 111

6.23 Allosteric Regulation of Enzymes

The hypothetical enzyme shown here has four subunits: two catalytic (blue), the other two regulatory (pink). When the enzyme is in its active form, the active sites on the catalytic subunits can accept substrate. When the enzyme is in its inactive form, the allosteric sites on the regulatory subunits can accept inhibitor.

We described the general effects of these factors on proteins in Chapter 3. Here, we will examine their effects on enzyme function, which, of course, depends on enzyme structure and chemistry.

No product formation I I Product formation

Enzymes and their environment

Enzymes enable cells to perform chemical reactions and carry out complex processes without using the extremes of temperature and pH employed by chemists in the laboratory. However, because of their three-dimensional structures and the chemistry of the side chains in their active sites, enzymes are highly sensitive to temperature and pH.

Q The first reaction is the commitment step.

Q Each of these reactions is catalyzed by a different enzyme, and each forms a different intermediate product.

NH₂

H—C — COO⁻ ■

I H—C — OH

I CH₃

Threonine

(starting material)

O

*> c—COO⁻

pH affects enzyme activity. The rates of most enzyme-catalyzed reactions depend on the pH of the medium in which they occur. Each enzyme is most active at a particular pH; its activity decreases as the solution is made more acidic or more basic than its "ideal" pH (Figure 6.25).

Several factors contribute to this effect. One is the ionization of carboxyl, amino, and other groups on either the substrate or the enzyme. In neutral or basic solutions, carboxyl groups (—COOH) release H⁺ to become negatively charged carboxylate groups (—COO⁻). Similarly, amino groups (—NH₂) accept H⁺ ions in neutral or acidic solutions, becoming positively charged —NH₃⁺ groups (see Chapter 2). Thus, in a neutral solution, a molecule with an amino group will be attracted electrically to another molecule that has a carboxyl group, because both groups are ionized and they have opposite charges.

If the pH changes, however, the ionization of these groups may change. For example, at a low pH (high H⁺ concentration), the excess H⁺ may react with the —COO⁻ to form COOH. If this happens, the group is no longer charged and cannot interact with other charged groups in the protein, so the folding of the protein is altered. If this occurs at the active site of an enzyme, the enzyme may no longer have the correct shape to bind to its substrate.

The process of evolution matches enzymes and their work environments. For example, the protein-digesting enzyme pepsin, found only in the stomach, works best at the very low pH values that prevail in the stomach after a meal. In contrast, salivary amylase works best at neutral pH, which is characteristic of the mouth.

NH,

t

O

CH,

CH,

α-Ketobutyrate

(intermediate product)

H—C—COO⁻ ► H—C—CH₃CH₂

CH₃

Isoleucine

(end product)

1 Q Buildup of the end product allosterically inhibits * the enzyme catalyzing the commitment step, thus shutting down its own production.

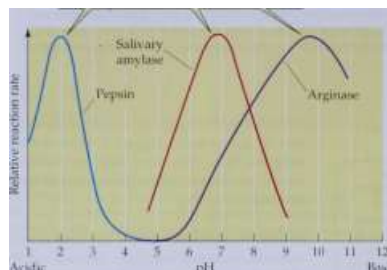
u

6.24 Inhibition of Metabolic Pathways

The commitment step is catalyzed by an allosteric enzyme that can be inhibited by the end product of the pathway. The specific pathway shown here is the synthesis of isoleucine, an amino acid, from threonine. This particular reaction series is performed by bacteria, but it is typical of many enzyme-catalyzed biological reactions.

112 CHAPTER SIX

Activity curves for three enzymes; each peaks at a pH where the enzyme is most effective.



6.25 pH Affects Enzyme Activity

Each enzyme catalyzes at a maximum rate at a particular pH.

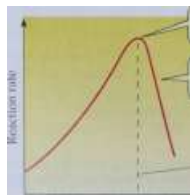
properties. Different isozymes within a given group may have different optimal temperatures.

The rainbow trout, for example, has several isozymes of the enzyme acetylcholinesterase, whose operation is essential to normal transmission of nerve impulses. If a rainbow trout is transferred from warm water to near-freezing water (2°C), the fish produces an isozyme of acetylcholinesterase that is different from the one it produces at the higher temperature. The new isozyme has a lower optimal temperature, which helps the fish to perform normally in the colder water.

In general, enzymes adapted to warm temperatures fail to denature because their tertiary structures are held together largely by covalent bonds, such as disulfide links, instead of the more heat-sensitive weak chemical forces. Most enzymes in humans are more stable at high temperatures than those of the bacteria that infect us, so that a moderate fever tends to denature bacterial enzymes, but not our own.

temperature affects enzyme activity. In general, warming increases the rate of an enzyme-catalyzed reaction, because at higher temperatures a greater fraction of the reactant molecules have enough energy to provide the activation energy for the reaction (Figure 6.26). Temperatures that are too high, however, inactivate enzymes, because at high temperatures enzyme molecules vibrate and twist so rapidly that some of their noncovalent bonds break. When heat destroys their tertiary structure, enzymes become inactivated, or denatured (see Chapter 3). Some enzymes denature at temperatures only slightly above that of the human body, but a few are stable even at the boiling or freezing points of water. All enzymes, however, show an optimal temperature for activity.

Individual organisms adapt to changes in the environment in many ways, one of which is based on groups of enzymes, called isozymes, that catalyze the same reaction but have different chemical compositions and physical



The reaction rate is maximal at the optimal temperature.

At higher temperatures, denaturation reduces enzyme activity.

Optimal temperature

Temperature

6.26 Temperature Affects Enzyme Activity

Each enzyme is most active at a particular optimal temperature.

Chapter Summary

Energy and Energy Conversions

- ▶ Energy is the capacity to do work. Potential energy is the energy of state or position; it includes the energy stored in chemical bonds. Kinetic energy is the energy of motion (and related forms such as electric energy, light, and heat).
- ▶ Potential energy can be converted to kinetic energy, which does work. Review Figure 6.1
- ▶ The first law of thermodynamics tells us that energy cannot be created or destroyed. The second law of thermodynamics tells us that, in any closed system, the quantity of energy available to do work (free energy) decreases and unusable energy (associated with entropy) increases. Review Figure 6.3
- ▶ Living things, like everything else, obey the laws of thermodynamics. Organisms are open systems that are part of a larger closed system. Review Figure 6.4
- ▶ Changes in free energy, total energy, temperature, and entropy are related by the equation $\Delta G = \Delta H - T\Delta S$.
- ▶ Spontaneous, exergonic reactions release free energy and have a negative ΔG . Nonspontaneous, endergonic reactions take up free energy and have a positive ΔG . Endergonic reactions proceed only if free energy is provided. Review Figure 6.5
- ▶ The change in free energy of a reaction determines its point of chemical equilibrium, at which the forward and reverse reactions proceed at the same rate. For spontaneous, exergonic reactions, the equilibrium point lies toward completion (the conversion of all reactants into products). Review Figure 6.6

ATP: Transferring Energy in Cells

- ▶ ATP (adenosine triphosphate) serves as an energy currency in cells. Hydrolysis of ATP releases a relatively large amount of free energy. Review Figure 6.8
- ▶ The ATP cycle couples exergonic and endergonic reactions, transferring free energy from the exergonic to the endergonic reaction. Review Figures 6.9, 6.10

Enzymes: Biological Catalysts

- ▶ The rate of a chemical reaction is independent of ΔG but is determined by the size of the activation energy barrier. Catalysts speed reactions by lowering the activation energy barrier. Review Figures 6.11, 6.12
- ▶ Enzymes are biological catalysts, proteins that are highly specific for their substrates. Substrates bind to the active site, where catalysis takes place, forming an enzyme-substrate complex. Review Figure 6.13
- ▶ At the active site, a substrate can be oriented correctly, chemically modified, or strained. As a result, the substrate readily forms its transition state, and the reaction proceeds. Review Figures 6.14, 6.15
- ▶ Substrate concentration affects the rate of an enzyme-catalyzed reaction. Review Figure 6.16

Molecular Structure Determines Enzyme Function

- ▶ The active site where substrate binds determines the specificity of an enzyme. Upon binding to substrate, some enzymes change shape, facilitating catalysis. Review Figures 6.13, 6.18
- ▶ Some enzymes require cofactors to carry out catalysis. Prosthetic groups are permanently bound to the enzyme. Coenzymes are not usually bound to the enzyme. They enter into the reaction as a "cosubstrate," as they are changed by the reaction and then released from the enzyme. Review Table 6.1 and Figure 6.19

Metabolism and the Regulation of Enzymes

- ▶ Metabolism is organized into pathways, in which the product of one reaction is a reactant for the next reaction. Each reaction is catalyzed by an enzyme.
- ▶ Enzyme activity is subject to regulation. Some compounds react irreversibly with enzymes and reduce their catalytic activity. Others react reversibly, inhibiting enzyme action only temporarily. A compound closely similar in structure to an enzyme's normal substrate may competitively inhibit the action of the enzyme. Review Figures 6.20, 6.21
- ▶ For allosteric enzymes, plots of reaction rate versus substrate concentration are sigmoidal, in contrast to plots of the same variables for non-allosteric enzymes. Review Figure

b.22

- ▶ Allosteric inhibitors bind to a site different from the active site and stabilize the inactive form of the enzyme. The multiple catalytic subunits of many allosteric enzymes interact cooperatively. Review Figure 6.23
- ▶ The end product of a metabolic pathway may inhibit the allosteric enzyme that catalyzes the commitment step of the pathway. Review Figure 6.24
- ▶ Enzymes are sensitive to their environment. Both pH and temperature affect enzyme activity. Review Figures 6.25, 6.26

For Discussion

1. How can endergonic reactions proceed in organisms?
2. Consider two proteins: One is an enzyme dissolved in the cytosol; the other is an ion channel in a membrane. Contrast the structures of the two proteins, indicating at least two important differences.
3. Plot free energy versus the course of an endergonic reaction and that of an exergonic reaction. Include the activation energy in both plots. Label E_a and ΔG on both graphs.
4. Consider an enzyme that is subject to allosteric regulation. If a competitive inhibitor (not an allosteric inhibitor) is added to a solution of such an enzyme, the ratio of enzyme molecules in the active form to those in the inactive form increases. Explain this observation.



Cellular Pathways

That Harvest Chemical Energy



The use of crushed plant materials, such as grapes and barley, to make alcoholic beverages, such as wine and beer, is as ancient as recorded history. But how these transformations come about has only recently been deciphered. The arts of the winemaker and brewer came under the scrutiny of science in the nineteenth century. Things came to a head (so to speak) when the German chemist Justus von Liebig claimed that these transformations were simply chemical reactions, and not some special property of the once-living plant material. Biologists, on the other hand, armed with microscopes and their cell theory, said that grape and barley extracts were converted to wine and beer by cells. Initially, these two ideas seemed to

conflict.

Louis Pasteur, the great French scientist, tackled the issue in 1860. A group of distillers wanted to use sugar beets, which grow abundantly in regions of France, to produce alcohol. With careful observations, Pasteur noted three things: (1) Nothing happened to the sugar beet mash unless tiny, living yeast cells were present; (2) in the presence of fresh air, yeast cells grew vigorously on the mash and bubbles of carbon dioxide were formed; and (3) without fresh air, the yeasts grew slowly, less carbon dioxide was produced, and alcohol was formed. Pasteur had shown that the production of alcohol by ground-up, sugary extracts was a property of living cells, thereby introducing the concept of biochemistry.

Biochemists eventually identified the intermediate substances in the pathway between sugar and carbon dioxide and showed that each intermediate step is catalyzed by a specific enzyme. This examples sums up the concept of metabolism: the sum total of all of the chemical transformations in living systems as they break down simple sugars and other molecules in order to liberate energy and build up complex molecules.

In this chapter, we will describe some aspects of metabolism, especially as they relate to the breakdown of sugars. The metabolism of sugars is important not only in making alcoholic beverages, but in providing the energy

Grape Harvest

The conversion of grape sugars into alcohol is mediated by enzymes in yeast cells.

that organisms store in ATP—the energy you use all the time to fuel both conscious actions such as turning the pages of this book, and automatic ones such as the beating of your heart.

Several principles govern metabolic pathways in the cell:

- ▶ Complex chemical transformations in the cell do not occur in a single reaction, but in a number of small steps that are connected in a pathway.
- ▶ Each reaction is catalyzed by a specific enzyme.
- ▶ Metabolic pathways are similar in all organisms.
- ▶ Many metabolic pathways are compartmentalized, with certain steps occurring inside an organelle.
- ▶ Metabolic pathways in organisms are regulated by the activities of a few enzymes.

Obtaining Energy and Electrons from Glucose

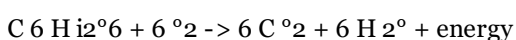
The most common fuel for living cells is the sugar glucose ($C_6H_{12}O_6$). Many other compounds serve as foods, but almost all of them are converted to glucose, or to intermediate compounds in the step-by-step metabolism of glucose. As you will see in this section, cells obtain energy from glucose by the chemical process of oxidation.

Cells trap free energy while metabolizing glucose

If glucose is burned in a flame, it readily forms carbon dioxide, water, and a lot of energy—but only if oxygen gas (O_2)



is present. The balanced equation for this combustion reaction is



(heat and light)

The same equation applies to the metabolism of glucose in cells, except that metabolism is a multi-step, controlled series of reactions, ending up with almost half of the energy captured in ATP.

The change in free energy (ΔG) for the complete conversion of glucose and oxygen to carbon dioxide and water, whether by combustion or by metabolism, is -686 kcal/mol ($-2,870 \text{ kJ/mol}$). Thus the overall reaction is highly exergonic and can drive the endergonic formation of a great deal of ATP from ADP and phosphate.

Some kinds of cells, unable to obtain or use oxygen gas, metabolize glucose incompletely, thus obtaining less ATP per glucose molecule. Not all of the carbon atoms of glucose are converted to carbon dioxide in this incomplete breakdown, which is called fermentation.

Three metabolic processes play roles in the utilization of glucose for energy: glycolysis, cellular respiration, and fermentation (Figure 7.1). All of these processes consist of metabolic pathways made up of many distinct, but coupled, chemical reactions.

- Glycolysis is a series of reactions that begins the metabolism of glucose in all cells and produces the three-carbon product pyruvate. A small amount of the energy stored in glucose is released in usable form.
- Cellular respiration occurs when the environment is aerobic (contains oxygen gas, O_2), and essentially converts pyruvate to carbon dioxide (CO_2). In the process, a great deal of the energy stored in the covalent bonds of pyruvate is released and trapped in ATP.
- Fermentation occurs when the environment is anaerobic (lacking oxygen gas). Instead of energy-poor CO_2 , relatively energy-rich molecules such as lactic acid or ethanol are produced, so the energy extracted from glucose is far less than under aerobic conditions.

AUTOTROPHS

Sun



Stored chemical energy

Food

AUTOTROPHS AND HETEROTROPHS

Glycolysis

Cellular respiration



CSS5SBP

Glycolysis

Pyruvate oxidation

I

Citric acid cycle

I

Respiratory chain

t

J

Fermentation reaction(s)

- Incomplete oxidation
- Waste products: Organic compound (lactic acid or ethanol and CO_2)
- Energy trapped: 2- $\{ATP\}$
- Complete oxidation
- Waste products: H_2O , CO_2
- Energy trapped: 36 v^{fe}

7.1 Energy for Life

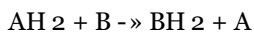
Both heterotrophic ("other feeding") and autotrophic ("self-feeding") organisms obtain energy from the food compounds that autotrophs produce by photosynthesis. They convert these compounds to glucose, then metabolize glucose by glycolysis, fermentation, and cellular respiration.

Redox reactions transfer electrons and energy

In Chapter 6, we described the addition of phosphate groups to ADP to make ATP as an endergonic reaction that can extract and store energy from exergonic reactions. Another way of transferring energy is to transfer electrons. A reaction in which one substance transfers one or more electrons to another substance is called an oxidation-reduction reaction, or redox reaction.

The gain of one or more electrons by an atom, ion, or molecule is called reduction. The loss of one or more electrons is called oxidation. Although oxidation and reduction are always defined in terms of traffic in electrons, we may also think in these terms when hydrogen atoms (not hydrogen ions) are gained or lost, because transfers of hydrogen atoms involve transfers of electrons ($H = H^+ + e^-$). Thus, when a molecule loses hydrogen atoms, it becomes oxidized:

oxidation



reduction

Oxidation and reduction always occur together: As one material is oxidized, the electrons it loses are transferred to another material, reducing that material. In a redox reaction, we call the reactant that becomes reduced an oxidizing agent and the one that becomes oxidized a reducing agent (Figure 7.2).

► An oxidizing agent accepts electrons; in the process of oxidizing the reducing agent, the oxidizing agent itself becomes reduced.

► Conversely, the reducing agent donates electrons; it becomes oxidized as it reduces the oxidizing agent.

116 CHAPTER SEVEN



B

Oxidized compound B

(oxidizing agent)

B is reduced, gaining electrons.

g Reduced © compound

7.2 Oxidation and Reduction Are Coupled

In a redox reaction, reactant A is oxidized and reactant B reduced. In the process, A loses electrons and B gains electrons. A proton may be transferred along with an electron, so that what is actually transferred is a hydrogen atom.

AH,

NAD⁺



BH₂

B

As compound AH₂ is oxidized, releasing two hydrogen atoms, NAD⁺ is reduced to NADH + H⁺.

Elsewhere, NADH + H⁺ reduces compound B to BH₂, at which time NADH is oxidized to NAD⁺.

7.4 NAD Is an Energy Carrier

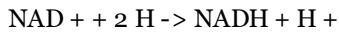
Thanks to its ability to carry free energy and electrons, NAD is a major and universal energy intermediary in cells.

In both the burning and the metabolism of glucose, glucose is the reducing agent and oxygen gas is the oxidizing agent. In a redox reaction, energy is transferred. Some of the energy originally present in the reducing agent becomes associated with the reduced product. (The rest remains in the reducing agent or is lost.) The overall AG of a redox reaction is negative. As we will see, some of the key reactions of glycolysis and cellular respiration are highly exergonic redox reactions.

The coenzyme NAD is a key electron carrier in redox reactions

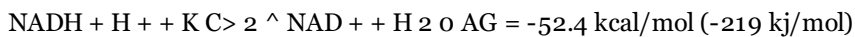
The main pair of oxidizing and reducing agents in cells is based on the compound NAD (nicotinamide adenine dinucleotide).

NAD exists in two chemically distinct forms, one oxidized (NAD⁺) and the other reduced (NADH + H⁺) (Figure 7.3). NAD⁺ and NADH + H⁺ participate in biological redox reactions. The reduction reaction



is formally equivalent to the transfer of two hydrogen atoms (2 H + 2 e⁻). However, what is actually transferred is a hydride ion (H⁻, a proton and two electrons), leaving a free proton (H⁺).

Oxygen gas (O₂) is highly electronegative (see Table 2.3) and readily accepts electrons from NADH. The oxidation of NADH + H⁺ by O₂ is highly exergonic:



f

Two hydrogen atoms (2 e⁻ + 2 H⁺) are released by another molecule.

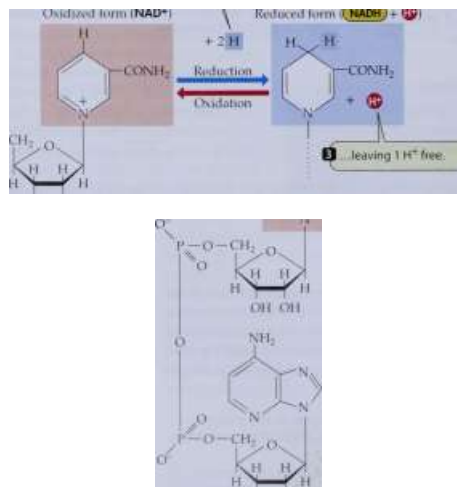
The

The ring structure of NAD acquires 2 e⁻ and 1 H⁺ + ...

Oxidized form (NAD⁺)

T

Reduced form (NADH + H⁺)



OH OH

7.3 Oxidized and Reduced Forms of NAD

NAD⁺ is the oxidized form and NADH the reduced form of NAD. The unshaded portion of the molecule remains unchanged by the redox reaction.

(Note that the oxidizing agent appears here as "O₂" instead of "O." This notation emphasizes that it is oxygen gas, O₂, that acts as the oxidizing agent.)

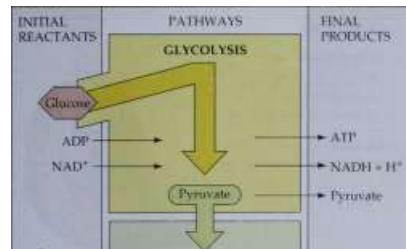
In the same way that ATP can be thought of as packaging free energy in bundles of about 12 kcal/mol (50 kJ/mol), NAD can be thought of as packaging free energy in bundles of approximately 50 kcal/mol (200 kJ/mol) (Figure 7.4).

NAD is not the only electron carrier in cells. As you will see, another carrier, FAD (flavin adenine dinucleotide), is also involved in transferring electrons during the metabolism of glucose.

An Overview: Releasing Energy from Glucose

The three energy-extracting processes of cells may be divided into distinct pathways:

► When O_2 is available as the final electron acceptor, four pathways operate. Glycolysis takes place first, and is



Glycolysis and cellular respiration

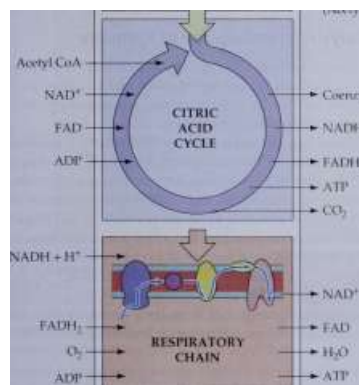
Pyruvate

Coenzyme A

NAD^+

PYRUVATE \rightarrow NAD^+ OXIDATION

• NAD^+ - Coenzyme A \rightarrow $NADH + H^+$



NAD^+ - CO_2

• $NADH + H^+$ \rightarrow Acetate (Acetyl CoA)

\rightarrow FAD \rightarrow H_2O

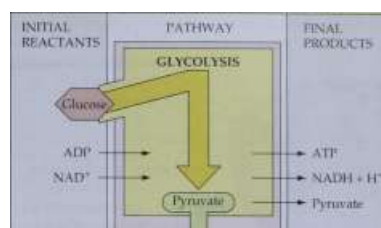
\rightarrow ATP

followed by the three pathways of cellular respiration: pyruvate oxidation, the citric acid cycle, and the respiratory chain. ► When O_2 is unavailable, pyruvate oxidation, the citric acid cycle, and the respiratory chain do not function, and fermentation is added to the glycolytic pathway.

These five chemical pathways, which we will consider one at a time, have different locations in the cell (Table 7.1). Figure 7.5 summarizes the starting reactants and products of these pathways.

In prokaryotes, the enzymes used in glycolysis, fermentation, and the citric acid cycle are soluble in the cytosol. The enzymes involved in pyruvate oxidation and the respiratory chain are associated with the inner surface of the plasma membrane or inward elaborations of that membrane (see Chapter 4).

Glycolysis and fermentation reactions



Pyruvate $NADH + H^+$

FERMENTATION REACTIONS

Lactate or alcohol

NAD^+ - NAD^+

-*- Lactate or alcohol + CO₂?

7.5 An Overview of the Cellular Energy Pathways

Energy-producing reactions can be grouped into five pathways: glycolysis, pyruvate oxidation, the citric acid cycle, the respiratory chain, and fermentation. The three middle pathways occur only in the presence of oxygen and are collectively referred to as cellular respiration.

In eukaryotes, glycolysis and fermentation take place in the cytoplasm outside of the mitochondria. The enzymes for these pathways were once believed to be soluble in the cytosol, but more recent discoveries suggest that at least some of them may be bound to components of the cyto-skeleton. The other reactions are associated with the mitochondria. Pyruvate oxidation and the respiratory chain are both associated with the inner membrane of mitochondria, where their enzymes are bound. The enzymes and reactions of the citric acid cycle are found in the mitochondrial matrix.

Glycolysis begins the breakdown of glucose. It is a sequence of ten separate chemical reactions in which glucose is incompletely oxidized to pyruvate. It contains an oxidative step in which the electron carrier NAD⁺ becomes reduced, acquiring electrons. The major products of glycolysis are ATP, pyruvate, and the electrons acquired by NAD. Both the pyruvate and the electrons must be processed further.

Cellular respiration operates when O₂ is available, yielding CO₂ and H₂O as products. It is made up of three pathways: pyruvate oxidation, the citric acid cycle, and the respiratory chain.

In pyruvate oxidation, the end product of glycolysis (pyruvate) is oxidized to acetate, which is activated by the addition of a coenzyme and further metabolized by the citric acid cycle.

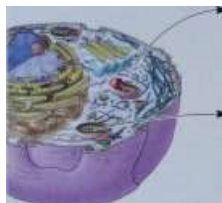
The citric acid cycle is a cyclic series of reactions in which the acetate becomes completely oxidized, forming CO₂ and transferring electrons (along with their hydrogen

118 CHAPTER SEVEN

/ 1 J Cellular Locations for Energy Pathways in Eukaryotes and Prokaryotes

EUKARYOTES

PROKARYOTES



External to mitochondrion

Glycolysis Fermentation

Inside mitochondrion

Inner membrane Pyruvate oxidation Respiratory chain

Matrix Citric acid cycle

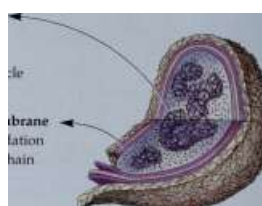
In cytoplasm

Glycolysis Fermentation Citric acid cycle

On inner face

of plasma membrane

Pyruvate oxidation Respiratory chain



nuclei) to carrier molecules. The citric acid cycle produces many more electrons than are produced in glycolysis. And, as we are about to see, harvesting more electrons means a greater ultimate harvest of ATP.

In glycolysis, pyruvate oxidation, and the citric acid cycle, the electron carriers NAD^+ and FAD become reduced and acquire hydrogen atoms. Through these pathways, energy originally present in the covalent bonds of glucose becomes associated with reduced forms of these carriers ($\text{NADH} + \text{H}^+$ and FADH_2).

The fourth energy-extracting pathway for aerobic cells is the respiratory chain, which releases energy from the reduced $\text{NADH} + \text{H}^+$ in such a way that it can be used to form ATP. This pathway consists of a series of redox reactions in which electrons derived from hydrogen atoms are passed from one type of carrier to another and finally are allowed to react with O_2 to produce water. Hydrogen is an outstanding fuel. When it reacts with O_2 , a great deal of free energy is released; better still, the "waste" product of this reaction—water—is not toxic to the environment or to any organism that produces it.

The transfer of electrons along the respiratory chain drives the active transport of hydrogen ions (protons) from the mitochondrial matrix into the space between the inner and outer mitochondrial membrane. This active transport sets up an imbalance of both concentration and charge across the membrane. As we saw in Chapter 6, such imbalances represent potential energy. This energy is recaptured by the subsequent diffusion of protons back into the matrix, which is coupled to the synthesis of ATP from ADP and P_i .

Overall, the inputs to the respiratory chain are hydrogen atoms and O_2 , and the outputs are water and energy captured as ATP.

In the discussion that follows, we will examine in more detail the four pathways of aerobic energy metabolism (glycolysis, pyruvate oxidation, the citric acid cycle, and the respiratory chain), and fermentation.

Glycolysis: From Glucose to Pyruvate

Glycolysis (also called the glycolytic pathway) takes place in the cytoplasm. It may be regarded as a common pathway to be followed either by cellular respiration or, under anaerobic conditions, fermentation.

In glycolysis, glucose is only partly oxidized. A molecule of glucose taken in by a cell enters the glycolytic pathway, which consists of 10 reactions that convert the six-carbon glucose molecule, step by step, into two molecules of the three-carbon compound pyruvate (pyruvic acid)* (Figure 7.7, which appears on pages 120-121.). These reactions are accompanied by the net formation of two molecules of ATP and by the reduction of two molecules of NAD^+ to two molecules of $\text{NADH} + \text{H}^+$. At the end of the glycolytic pathway, then, energy has been transferred to ATP, and four hydrogen atoms have been transferred to $\text{NADH} + \text{H}^+$.

Glycolysis can be divided into two groups of reactions: energy-investing reactions that use ATP, and energy-harvesting reactions that produce ATP.

The energy-investing reactions of glycolysis require ATP

Using Figure 7.7, let us work our way through the glycolytic pathway. The first five reactions are endergonic; that is, the cell is investing free energy rather than gaining it during the early reactions of glycolysis. In separate reactions, two molecules of ATP are invested in attaching two phosphate groups to the sugar molecule (reactions 1 and 3), thereby raising its free energy by about 15 kcal/mol (62.7 kJ/mol) (Figure 7.6). Later, these phosphate groups will be transferred to ADP to make new molecules of ATP.

*We tend to use words like "pyruvate" and "pyruvic acid" interchangeably. However, at the pH values commonly found in cells, the ionized form—pyruvate—is present rather than the acid—pyruvic acid. Similarly, all carboxylic acids are present as ions (the "-ate" forms) at these pH values.

GLYCOLYSIS

<

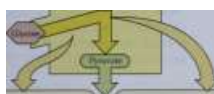
u

C

01

01

c U



The first reactions of glycolysis are all slightly endergonic.

These reactions are the "energy-harvesting" portion of glycolysis.

$$\text{R}-\text{C}-\text{H} + (\text{O}) \rightarrow \text{R}-\text{C}-\text{OH}$$

Since this is an oxidation reaction, it is very exergonic.

The third step, the formation of a phosphate ester (BPG) from an acid

O O O

R—C

Although both of these first steps of glycolysis use ATP as one of their substrates, each is catalyzed by a different, specific enzyme. The enzyme hexokinase catalyzes reaction 1, in which a phosphate group from ATP is attached to the six-carbon glucose molecule, forming glucose 6-phosphate. (A kinase is any enzyme that catalyzes the transfer of a phosphate group from ATP to another substrate.) In reaction 2, the six-membered glucose ring is rearranged into a five-membered fructose ring. Then, in reaction 3, the enzyme phosphofructokinase adds a second phosphate (taken from another ATP) to the fructose ring, forming a six-carbon sugar bisphosphate.*

*The root bis- means "two." A sugar bisphosphate has two phosphate groups attached to two different carbons, as opposed to the prefix di-, which implies the serial attachment of two phosphate groups to one carbon, as in ADP (adenosine diphosphate).

OH+ HPO;" ^R—c

4

O—P-O"

O" + H 2 o

is slightly endergonic, but not nearly enough to offset the drop in free energy from the oxidation.

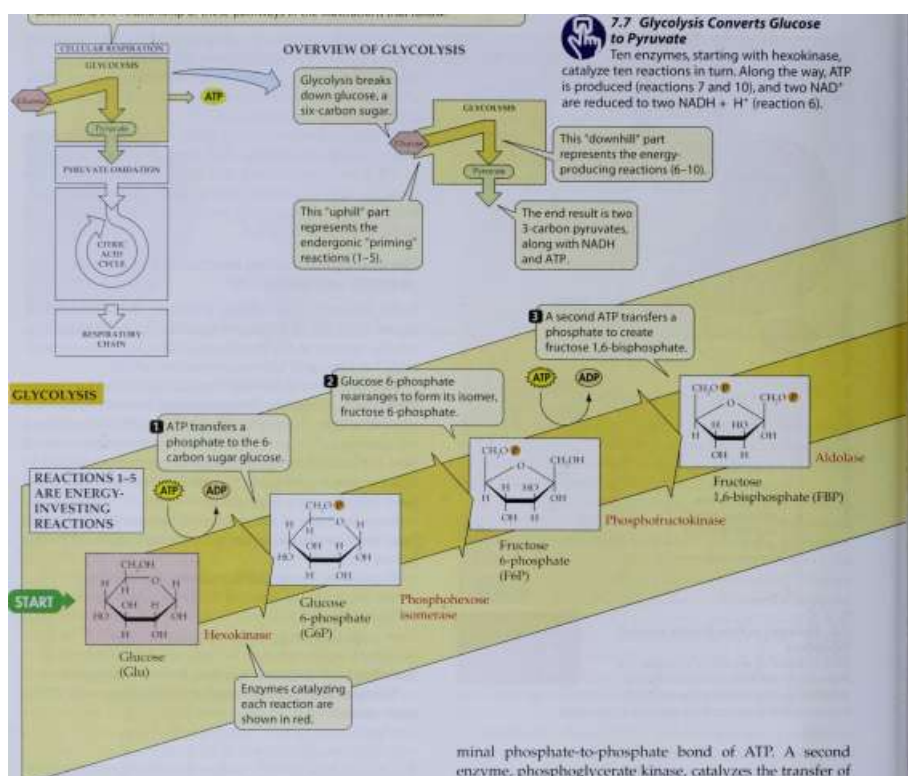
If this big energy drop were simply a loss of heat, glycolysis would not provide useful energy to the cell. However, rather than being lost, this energy is stored by reducing two molecules of NAD + to make two molecules of NADH + H + This stored energy is regained later—either in the respiratory chain, by the formation of ATP, or in the last step of fermentation, in which pyruvate or its product is reduced by the two molecules of NADH + H + , forming NAD + . Be-cause NAD + is present in small amounts in the cell, it must be recycled to keep glycolysis going; if none of the NADH is oxidized back to NAD + , glycolysis comes to a halt.

The remaining steps of glycolysis are simpler. The two phosphate groups of BPG are transferred, one at a time, to

The pathways of glycolysis and cellular respiration (pyruvate oxidation, citric acid cycle and respiratory chain) are represented by a "road map" of symbols that guide you to better understand the relationship of these pathways in the illustrations that follow.

7.7 Glycolysis Converts Glucose to Pyruvate

Ten enzymes, starting with hexokinase, catalyze ten reactions in turn. Along the way, ATP is produced (reactions 7 and 10), and two NAD + are reduced to two NADH + H + (reaction 6).



molecules of ADP, with a rearrangement in between. More than 20 kcal (83.6 kJ/mol) of free energy is stored in ATP for every mole of BPG broken down. Finally, we are left with two moles of pyruvate for every mole of glucose that entered glycolysis.

substrate-level phosphorylation. The enzyme-catalyzed transfer of phosphate groups from donor molecules to ADP molecules (reaction 7) is called substrate-level phosphorylation. This process is driven by energy obtained from oxidation. For example, when G3P reacts with a phosphate group (P_i) and NAD⁺, becoming BPG, an aldehyde is oxidized to a carboxylic acid, with NAD⁺ acting as the oxidizing agent. The oxidation provides so much energy that the newly added phosphate group is linked to the rest of the molecule by a bond that has even more energy than the terminal phosphate-to-phosphate bond of ATP. A second enzyme, phosphoglycerate kinase, catalyzes the transfer of this phosphate group from BPG to ADP in reaction 7, forming ATP. Both reactions are exergonic, even though a substantial amount of energy is consumed in the formation of ATP.

minimal phosphate-to-phosphate bond of ATP. A second enzyme, phosphoglycerate kinase, catalyzes the transfer of this phosphate group from BPG to ADP in reaction 7, forming ATP. Both reactions are exergonic, even though a substantial amount of energy is consumed in the formation of ATP.

GLYCOLYSIS MAY BE FOLLOWED BY FERMENTATION. A review of

the glycolytic pathway shows that at the beginning of glycolysis, two molecules of ATP are used per molecule of glucose, but that ultimately four molecules of ATP are produced (two for each of the two BPG molecules)—a net gain of two ATP molecules and two NADH + H⁺.

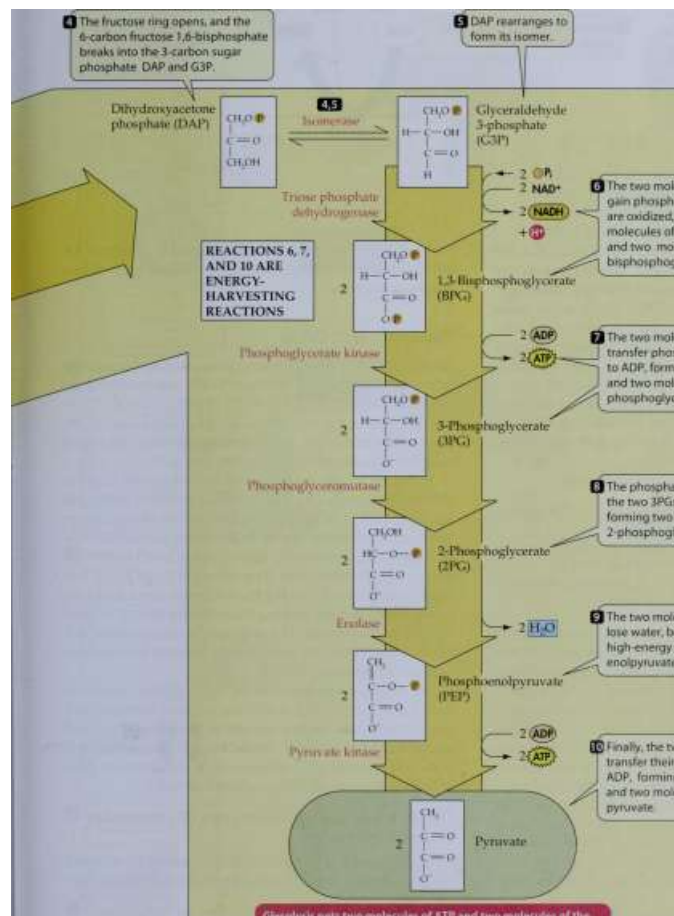
When fermentation follows glycolysis, the total usable energy yield is just these two ATP molecules per glucose molecule. Under anaerobic conditions, the NADH + H⁺ is rapidly recycled to NAD⁺ by the reduction of pyruvate. The NAD⁺ is then available for the glycolytic reaction catalyzed by the enzyme triose phosphate dehydrogenase (reaction 6 in Figure 7.7).

CELLULAR PATHWAYS THAT HARVEST CHEMICAL ENERGY 121

| The fructose ring opens, and the 6-carbon fructose 1,6-bisphosphate breaks into the 3-carbon sugar phosphate DAP and G3P.

The two molecules of G3P gain phosphate groups and are oxidized, forming two molecules of NADH + H⁺ and two molecules of 1,3-bisphosphoglycerate (BPG).

The two molecules of BPG transfer phosphate groups to ADP, forming two ATPs and two molecules of 3-phosphoglycerate (3PG).



J^jThe phosphate groups on the two 3PGs move, forming two 2-phosphoglycerates (2PG).

Q The two molecules of 2PG lose water, becoming two high-energy phospho-^j enolpyruvates (PEP).

C\$ Finally, the two PEPs

transfer their phosphates to ADP, forming two ATPs and two molecules of pyruvate.

Glycolysis nets two molecules of ATP and two molecules of the electron carrier NADH. Two molecules of pyruvate are produced.

On the other hand, in the presence of oxygen, eukaryotes and some bacteria reap far more energy by completely oxidizing pyruvate and by oxidizing $\text{NADH} + \text{H}^+$ through

the respiratory chain, as we will see in the sections that follow. In eukaryotes, these reactions take place in the mitochondria.

122 CHAPTER SEVEN

©.

t

Pyruvate is oxidized to the acetyl group, with the release of CO_2 .

7.8 The Pyruvate Dehydrogenase Complex Catalyzes Pyruvate Oxidation

A massive multiprotein complex, pyruvate dehydrogenase converts pyruvate to acetyl CoA by transferring electrons, removing a carboxyl group, and adding a coenzyme (CoA). Energy is stored temporarily in acetyl CoA.

Pyruvate Oxidation

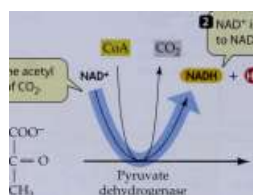
The oxidation of pyruvate to acetate is the link between glycolysis and cellular respiration. Pyruvate oxidation is a multistep reaction catalyzed by an enormous enzyme complex that is attached to the inner mitochondrial membrane. Pyruvate diffuses into the mitochondrion, where it is oxidized. In this reaction, the pyruvate, a three-carbon compound, loses two hydrogen atoms and a carboxyl group ($-\text{COO}^-$). The reaction yields the two-carbon acetyl group, as well as free energy and CO_2 . The free energy is captured when the acetyl group is linked to a coenzyme, called coenzyme A (CoA), producing acetyl coenzyme A (acetyl CoA) (Figure 7.8). Acetyl CoA has 7.5 kcal/mol (31.4 kJ/mol) more energy than simple acetate. (Acetyl CoA can donate the acetyl group to acceptors such as oxaloacetate, much as ATP can donate phosphate groups to various acceptors.)

There are three steps in this oxidation reaction:

- Pyruvate is oxidized to the acetyl group, and CO_2 is released.
- Part of the energy from the oxidation in the first step is saved by the reduction of NAD^+ to $\text{NADH} + \text{H}^+$.
- Some of the remaining energy is stored temporarily by the combining of the acetyl group with CoA.

An analogous three-step reaction occurs in reaction 6 of the glycolytic pathway, when G3P is converted to BPG (see Figure 7.6). In that reaction, an aldehyde group is oxidized to an acid, some of the energy released by oxidation is stored in $\text{NADH} + \text{H}^+$, and some of the remaining energy is preserved in a second phosphate bond in the BPG molecule. As the similarity between these two three-step reactions shows, a good metabolic idea is likely to appear more than once; we will see it again in the citric acid cycle.

As you might suspect, a complex set of steps such as those in pyruvate oxidation requires more than one type of catalytic protein. This reaction is catalyzed by the pyruvate dehydrogenase complex, a huge multi-protein machine that consists of 72 polypeptide chains—24 each of three differ-



NAD^+ is reduced to NADH and H^+

Coenzyme A is added to

the acetyl group,

forming acetyl CoA.

$\text{C}-\text{CoA}$

O

Pyruvate

Pyruvate

dehydrogenase

complex

CH 3 Acetyl CoA

ent protein molecules, for a total molecular weight of 4.6 million. The three component enzymes use a total of five different coenzymes.

The Citric Acid Cycle

Acetyl CoA is the starting point for the citric acid cycle (also called the Krebs cycle or the tricarboxylic acid cycle) (Figure 7.9). This pathway, which consists of eight reactions, completely oxidizes the two-carbon acetyl group to two molecules of carbon dioxide. The free energy released from these reactions is captured by NAD, FAD, and ADP

As Figure 7.7 shows, the metabolism of glucose to pyruvate is accompanied by a drop in free energy of about 140 kcal/mol (585 kJ/mol). About a third of this energy is captured in the formation of ATP and reduced NAD ($\text{NADH} + \text{H}^+$). Oxidizing pyruvate to acetate yields much additional free energy. The citric acid cycle takes the acetyl group and breaks it down to CO_2 , using the hydrogen atoms to reduce carrier molecules and passing chemical free energy to those carriers in the process. The reduced carriers are later oxidized in the respiratory chain, which transfers an enormous amount of free energy to ATP.

The principal inputs to the citric acid cycle are acetate (in the form of acetyl CoA), water, and oxidized electron carriers. The principal outputs are carbon dioxide and reduced electron carriers. Overall, for each acetyl group, the citric acid cycle removes two carbons as CO_2 and uses four pairs of hydrogen atoms to reduce carrier molecules.

The citric acid cycle produces two CO_2 molecules and reduced carriers

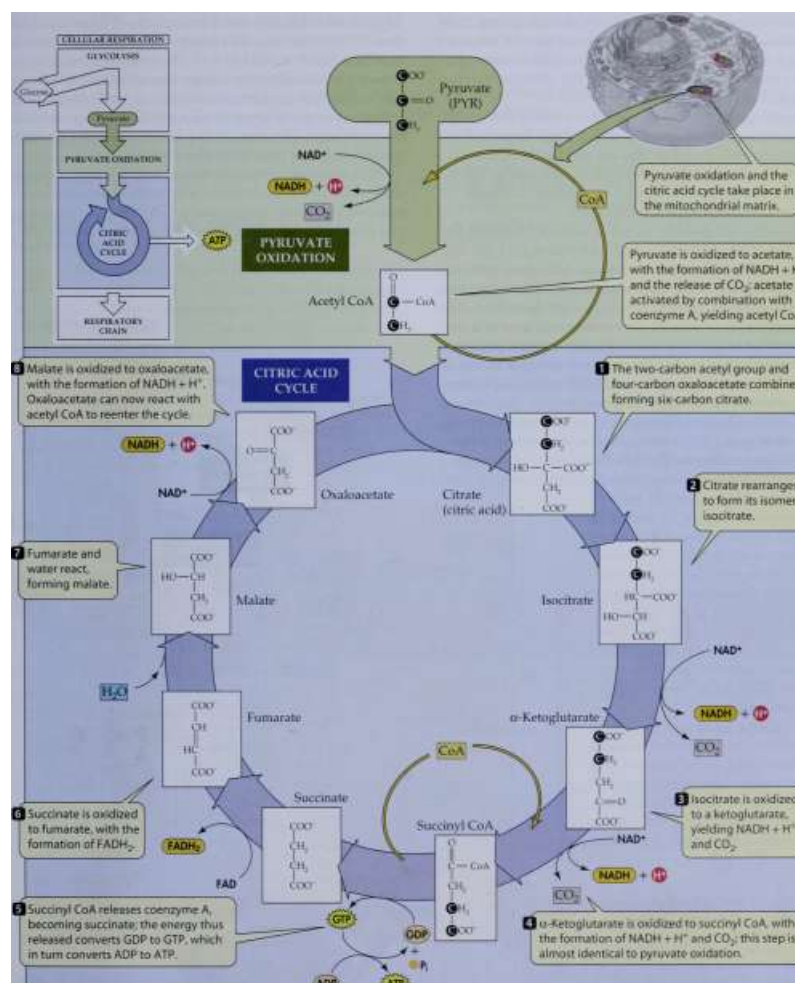
At the beginning of the citric acid cycle, acetyl CoA, which has two carbon atoms in its acetyl group, reacts with a four-

©.

7.9 The Citric Acid Cycle

Pyruvate diffuses into the mitochondrion and is oxidized to acetyl CoA, which enters the citric acid cycle. The two carbons from acetyl CoA are shown in black circles. Reactions 3, 4, 6, and 8 accomplish the major overall effects of the cycle—the trapping of energy—by passing electrons to NAD or FAD. Reaction 5 traps energy directly in ATP.

CELLULAR PATHWAYS THAT HARVEST CHEMICAL ENERGY 123



(Succinyl CoA releases coenzyme A, becoming succinate; the energy thus released converts GDP to GTP, which in turn converts ADP to ATP.

α -Ketoglutarate is oxidized to succinyl CoA, with the formation of $\text{NADH} + \text{H}^+$ and CO_2 ; this step is almost identical to pyruvate oxidation.

124 CHAPTER SEVEN

carbon acid, oxaloacetate, to form the six-carbon compound citrate (citric acid). The remainder of the cycle consists of a series of enzyme-catalyzed reactions in which citrate is degraded to a new four-carbon molecule of oxaloacetate. This new oxaloacetate can react with a second acetyl CoA, producing a second molecule of citrate and thus enabling the cycle to continue. Acetyl CoA enters the cycle from pyruvate oxidation, and CO_2 exits.

The citric acid cycle is maintained in a steady state—that is, although materials enter and leave and intermediate compounds are formed as they are metabolized, the concentrations of molecules in the cycle do not change much. Pay close attention to the numbered reactions in Figure 7.9 as you read the next several paragraphs.

The energy temporarily stored in acetyl CoA drives the formation of citrate from oxaloacetate (reaction 1). During this reaction, the coenzyme A molecule falls away, to be recycled. In reaction 2, the citrate molecule is rearranged to form isocitrate. In reaction 3, a CO_2 molecule and two hydrogen atoms are removed, converting isocitrate to α -ketoglutarate. As Figure 7.10 indicates, this reaction produces a large drop in free energy. The released energy is stored in $\text{NADH} + \text{H}^+$ and can be recovered later in the respiratory chain, when the $\text{NADH} + \text{H}^+$ is reoxidized.

Like the oxidation of pyruvate to acetyl CoA, reaction 4 of the citric acid cycle is complex. The five-carbon α -ketoglutarate molecule is oxidized to the four-carbon molecule succinate. In the process, CO_2 is given off, some of the oxidation energy is stored in $\text{NADH} + \text{H}^+$, and some of the energy is preserved temporarily by combining succinate with CoA to form succinyl CoA. In reaction 5, the energy in succinyl CoA is harvested to make GTP (guanosine triphosphate) from GDP and P_i , which is another example of substrate-level phosphorylation. GTP is then used to make ATP from ADP.

Free energy is released in reaction 6, in which the succinate released from succinyl CoA in reaction 5 is oxidized to fumarate. In the process, two hydrogens are transferred to an enzyme that contains the carrier FAD. After a molecular rearrange-

"3 -100

J4

C

u <

bC

c

01

-200

-300

ΔG°

C re X U

-500

7.10 The Citric Acid Cycle Releases Much More Free Energy Than Glycolysis Does

Electron carriers (NAD in glycolysis; NAD and FAD in the citric acid cycle) are reduced and ATP is generated in reactions coupled to other reactions, producing major drops in free energy as metabolism proceeds.

-600

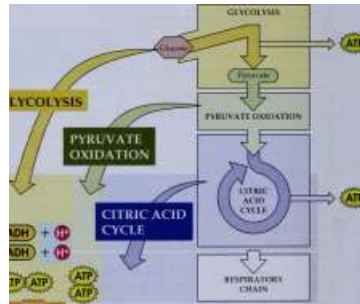
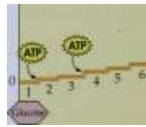
-700

ment (reaction 7), one more NAD^+ reduction occurs, producing oxaloacetate from malate (reaction 8). These two reactions illustrate a common biochemical mechanism: Water (H_2O) is added in reaction 7 to form an $-\text{OH}$ group, and then the H from that $-\text{OH}$ group is removed in reaction 8 to reduce NAD^+ to $\text{NADH} + \text{H}^+$. Essentially, these two reactions provide energy by using a very abundant substance (H_2O). The final product, oxaloacetate, is ready to combine with another acetyl group from acetyl CoA and go around the cycle again. The citric acid cycle operates twice for each glucose molecule that enters glycolysis.

Although most of the enzymes of the citric acid cycle are dissolved in the mitochondrial matrix, there are two exceptions: succinate dehydrogenase, which catalyzes reaction 6, and α -ketoglutarate dehydrogenase, which catalyzes reac-

muHMFrii-m.gnBia

GLYCOLYSIS



(nadh) + ©

. (NADH) + (\$

9 10 y (nadh) + CD

(Pyruvate)



Acetyl-CoA—* 1 a



Citrate

;nadh) + <jp

L_r (nadh) + (JP

(NADH) +(JP

(FADH 2) (FADH 2)



(nadh) + <3> (nadh) + (JP

tt

Oxaloacetate

tion 4. These enzymes are integral membrane proteins of the inner mitochondrial membrane.

Generations of students have asked the question, "Why did this complicated system evolve to achieve the simple goal of oxidizing two carbon acetyl units to CO_2 ?" There are two reasons. First, the cycle includes molecules that have other roles in the cell. As we will see later in this chapter, the intermediates of the citric acid cycle are themselves catabolic (breakdown) products or anabolic (synthesis) sources of other molecules, such as amino acids and nucleotides. Second, the citric acid cycle is far more efficient at tapping off energy than any single reaction could be.

The Respiratory Chain: Electrons, Proton Pumping, and ATP

Without NAD⁺ and FAD, the oxidative steps of glycolysis, pyruvate oxidation, and the citric acid cycle could not occur. Once reduced, these carriers must have some place to donate their hydrogens ($\text{H}^+ + \text{e}^-$). The fate of these protons and electrons is the rest of the story of cellular respiration. The story has three parts:

- First, the electrons pass through a series of membrane-associated electron carriers called the respiratory chain.
- Second, the flow of electrons along the chain causes the active transport of protons across the inner mitochondrial membrane, out of the matrix, creating a concentration gradient.
- Third, the protons diffuse back into the mitochondrial matrix through a proton channel, which couples this diffusion to the synthesis of ATP.

The overall process of ATP synthesis resulting from electron transport through the respiratory chain is called oxidative phosphorylation.

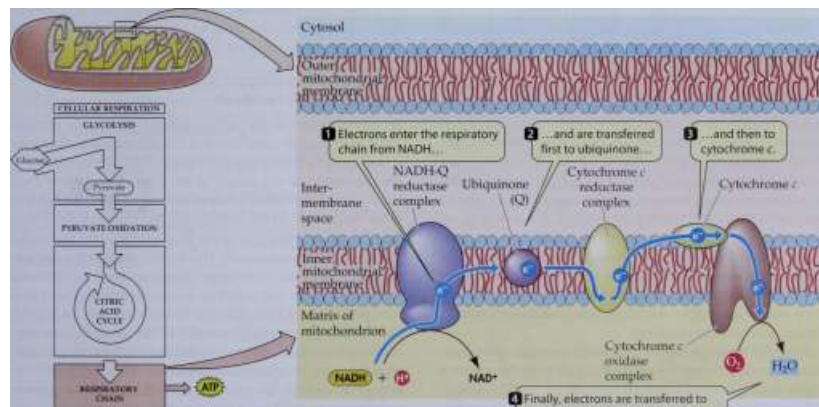
The respiratory chain transports electrons and releases energy

The respiratory chain contains three components:

- ▶ Three large protein complexes, containing carrier molecules and their associated enzymes
- ▶ A small protein called cytochrome c
- ▶ A nonprotein component called ubiquinone (abbreviated Q)

The large protein complexes are bound to the folds of the inner mitochondrial membranes, the cristae (see Figure 4.16), in eukaryotes, or to the plasma membrane of aerobic prokaryotes. Cytochrome c is a peripheral membrane protein that lies in the space between the inner and outer mitochondrial membranes, loosely attached to the inner membrane. Ubiquinone (Q) is a small, nonpolar molecule that moves freely within the hydrophobic interior of the phospholipid bilayer of the inner membrane (Figure 7.11).

NADH + H⁺ passes hydrogens to Q by way of the first large protein complex, NADH-Q reductase, which contains 26 polypeptides and attached prosthetic groups.



©

7.1 7 The Oxidation of NADH + H⁺

Electrons from NADH + H⁺ are passed through the respiratory chain, a series of carrier molecules in the inner mitochondrial membrane. The carriers gain

Cytochrome c

oxidase

complex

Q Finally, electrons are transferred to molecular oxygen, which picks up protons and electrons to form water.

free energy when they become reduced and release free energy when they are oxidized.

126 CHAPTER SEVEN

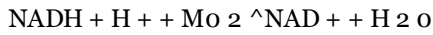
NADH-Q reductase passes the hydrogens to Q, forming QH₂. Cytochrome c reductase, with 10 subunits, receives hydrogens from QH₂ and passes them to cytochrome c. Cytochrome c oxidase, with 8 subunits, receives electrons from cytochrome c and passes them to oxygen. Reduced oxygen ($\frac{1}{2} \text{O}_2$) picks up two hydrogen ions (H⁺) to form H₂O. Different subunits within each of these large protein complexes bear different electron carriers, so electrons are transported within each complex, as well as from complex to complex.

The electron carriers of the respiratory chain (including those contained in the three protein complexes) differ as to how they change when they become reduced. NAD⁺, for example, accepts H⁻ (a hydride ion—one proton and two electrons), leaving the proton from the other hydrogen atom to float free: NADH + H⁺. Other carriers, including Q, bind both protons and both electrons, becoming, for example, QH₂. The remainder of the chain, however, is only an electron transport process. Electrons, but not protons, are passed from Q to cytochrome c. An electron from QH₂ reduces a cytochrome's Fe³⁺ to Fe²⁺.

Electrons pour into the pool of Q molecules from the NADH + H⁺ pathway, and some come from another source: the succinate-to-fumarate reaction of the citric acid cycle (reaction 6 in Figure 7.9). Another protein complex, succinate-Q reductase, links the oxidation of succinate to the reduction of Q (Figure 7.12). The enzyme that constitutes the first part of succinate-Q reductase has attached to it an FAD carrier molecule, which is reduced by succinate to FADH₂. Later, hydrogen atoms are transferred to the Q molecules. No protons are pumped, and hence no ATP is generated in the succinate-to-Q

branch of the respiratory chain.

Why should the respiratory chain have so many links? Why, for example, don't cells just use the following single step?



Well, to begin with, no enzyme will catalyze the direct oxidation of $\text{NADH} + \text{H}^+$ by oxygen. More fundamentally, this would be an untamable reaction. It would be terrifically ex-ergonic—rather like setting off a stick of dynamite in the cell. There is no biochemical way to harvest that burst of energy efficiently and put it to physiological use (that is, no metabolic reaction is so endergonic as to consume a significant fraction of that energy in a single step). To control the release of energy during oxidation of glucose in a cell, evolution has produced the lengthy respiratory chain we observe today: a series of reactions, each releasing a small, manageable amount of energy.

Electron transport within each of the three protein complexes results, as we'll see, in the pumping of protons across the inner mitochondrial membrane, and the return of the protons across the membrane leads to the formation of ATP. Thus the energy originally contained in glucose and other foods is finally tucked into the cellular energy currency, ATP. For each pair of electrons passed along the res-

Electrons from $\text{NADH} + \text{H}^+$ are accepted by NADH-Q reductase at the start of the respiratory chain.



u

^

O

o

>

13 "a!

aj

e

oo

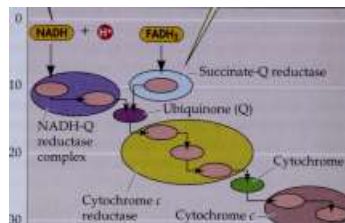
c 3

u

Electrons also come from succinate by way of FADH_2 ; these electrons are accepted by succinate-Q reductase rather than by NADH-Q reductase.

$(\text{NADH}) + \text{O}_2$

Succinate-Q reductase



-40

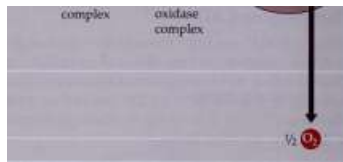
-50

Cytochrome c

reductase Cytochrome c -

complex oxidase

complex



7.12 The Complete Respiratory Chain

Electrons enter the chain from two sources, but they follow the same pathway from Q onward.

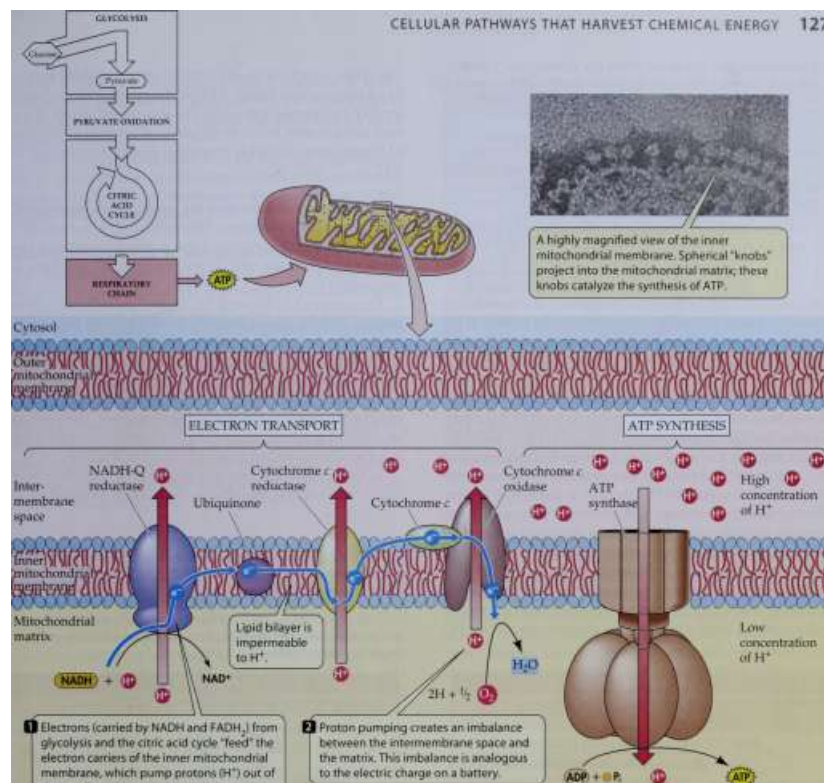
piratory chain from NADH + H + to oxygen, three molecules of ATP are formed.

If only electrons are carried through the final reactions of the respiratory chain, what happens to H + ? And how is electron transport coupled to ATP production?

Active proton transport is followed by diffusion coupled to ATP synthesis

As we have seen, all the carriers and enzymes of the respiratory chain (except cytochrome c) are embedded in the inner mitochondrial membrane (see Figure 7.11). The operation of the respiratory chain results in the active transport of protons (H +) against their concentration gradient, across the inner membrane of the mitochondrion from inside to outside ("outside" being the space between the inner and outer mitochondrial membranes). This occurs because the carriers are arranged on the three large complexes such that protons are produced on one side (the intermembrane space) and transported along with electrons to the other side (facing the mitochondrial matrix) (Figure 7.13). Because of the charge on the proton (H +), this transport causes not only a difference in proton concentration, but also a difference in electric charge across the membrane, with the inside of the organelle (the matrix) more negative than the outside.

Together, the proton concentration gradient and the charge difference constitute a source of potential energy called the proton-motive force. This force tends to drive the



(nadh) + (jp

Electrons (carried by NADH and FADH₂) from glycolysis and the citric acid cycle "feed" the electron carriers of the inner mitochondrial membrane, which pump protons (H +) out of the matrix to the space between inner and outer membranes.

o Proton pumping creates an imbalance between the intermembrane space and the matrix. This imbalance is analogous to the electric charge on a battery.

(ADP) +

A.

7.13 A Chemiosmotic Mechanism Produces ATP

As electrons pass through the series of carriers in the respiratory chain, protons are pumped from the mitochondrial matrix into the intermembrane space. As the protons return to the matrix through an ATP synthase, ATP forms.

protons back across the membrane, just as the charge on a battery drives the flow of electrons, discharging the battery. The conversion of the proton-motive force into kinetic energy is prevented by the fact that the lipid bilayer of the inner membrane is impermeable to protons. However, they can diffuse across the membrane by passing through a specific channel protein, called ATP synthase, that couples proton movement to the synthesis of ATP. This coupling of

§J Because of the imbalance, protons return to the matrix by passing through an ATP synthase in the inner membrane. This "relaxation" of the proton imbalance is coupled with the formation of ATP in the complex.

proton-motive force and ATP synthesis is called the chemiosmotic mechanism.

THE CHEMIOSMOTIC MECHANISM COUPLES ELECTRON TRANSPORT TO

atp synthesis. The chemiosmotic mechanism has three parts:

► The flow of electrons from NADH (or FADH₂) from one electron carrier to another in the respiratory chain is a series of exergonic reactions that occurs in the inner mitochondrial membrane.

128 CHAPTER SEVEN

► These exergonic reactions drive the endergonic pumping of H⁺ out of the mitochondrial matrix and across the inner membrane into the intermembrane space. This pumping forms a H⁺ gradient.

► The potential energy of the H⁺ gradient, or proton-motive force, is harnessed by ATP synthase. This protein has two roles: It acts as a channel allowing the H⁺ to diffuse back into the matrix, and it uses the energy of that diffusion to make ATP from ADP and P_i ; .

ATP synthesis is a reversible reaction, and ATP synthase can also act as an ATPase, hydrolyzing ATP to ADP and P_i

$\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_i + \text{free energy}$

If the reaction goes to the right, free energy is released, and is used to pump H⁺ out of the mitochondrial matrix. If the reaction goes to the left, it uses free energy from H⁺ diffusion into the matrix to make ATP. What makes it prefer ATP synthesis?

There are two answers to this question. First, ATP is removed from the mitochondrial matrix as soon as it is made, keeping the ATP concentration in the matrix low and driving the reaction toward the left. A person hydrolyzes about 10²⁵ ATP molecules per day, and clearly the vast majority are recycled. Second, the H⁺ gradient is constantly replenished by electron pumping. (The electrons, you recall, come from the oxidation of NADH, which itself gets reduced by the oxidations of glycolysis and the citric acid cycle. So, one reason for eating food is to replenish the H⁺ gradient!)

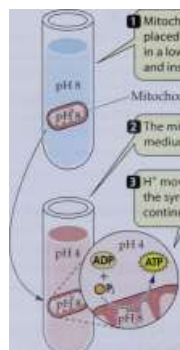
ATP synthase is a large multi-protein machine, having 16 different polypeptides in mammals. It has two visible and functional parts. One is the membrane channel for H⁺ .

EXPERIMENT 1

Question: Can an H⁺ gradient drive ATP synthesis by isolated mitochondria?

Q Mitochondria are isolated from cells and X placed in a medium at pH 8. This results ^^ in a low H⁺ concentration both outside and inside the organelles.

Mitochondrion



The mitochondria are moved to an acidic medium (pH 4; high H⁺ concentration).

H⁺ movement into mitochondria drives the synthesis of ATP in the absence of continuous electron transport.

Conclusion: In the absence of electron transport, an artificial H⁺ gradient is sufficient for ATP synthesis by mitochondria.

The other, which sticks out into the mitochondrial matrix like a lollipop (see Figure 7.13), is the actual ATP synthesis (or ATP hydrolysis) active site. The actual mechanism of energy transduction involves the physical rotation of the core of the enzyme, with this rotational energy transferred to ATP.

TWO EXPERIMENTS DEMONSTRATE THE CHEMIOSMOTIC MECHANISM.

Two key experiments have shown that (1) a proton (H^+) gradient and proton-motive force across a membrane can drive ATP synthesis; and (2) the enzyme ATP synthase is the catalyst for it.

Experiment 1 in Figure 7.14 "fooled" mitochondria into making ATP by raising the H^+ concentration in their environment. A sample of isolated mitochondria with a low H^+ concentration was suddenly put in an acidic medium with a high concentration of H^+ . The outer mitochondrial membrane, unlike the inner one, is freely permeable to H^+ , so H^+ rapidly diffused into the intermembrane space. This created an artificial gradient across the inner membrane, which the mitochondrion used to make ATP from ADP and P_i .

In Experiment 2, a proton pump from a bacterium was added to artificial membrane vesicles. It proceeded to

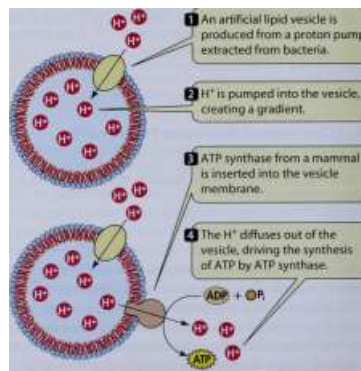
7.14 Two Experiments Demonstrate the Chemiosmotic Mechanism

These two experiments show that an H^+ gradient across a membrane is all that is needed to drive the synthesis of ATP by the enzyme ATP synthase. Whether the H^+ gradient is produced by the electron transport chain found in nature, or artificially as in these experiments, does not matter.

EXPERIMENT 2

Question: What is the role of H^+ pumps in ATP synthesis?

An artificial lipid vesicle is produced from a proton pump extracted from bacteria.



Conclusion: If an H^+ gradient is created by directional pumping, a second pump, acting as an H^+ channel, is necessary for ATP synthesis.

pump H^+ into the vesicles, creating a gradient. If mammalian ATP synthase was then put into the membranes of these vesicles, it made ATP even in the absence of the usual electron carriers.

These experiments show that the key to ATP synthesis is the H^+ gradient; it does not matter whether this gradient is produced naturally by the electron transport chain, or artificially by an experimenter.

PROTON DIFFUSION CAN BE UNCOUPLED FROM ATP PRODUCTION.

For the chemiosmotic mechanism to work, the diffusion of H^+ and the formation of ATP must be tightly coupled; that is, the protons must pass through the ATP synthase channel in order to move inward. If a simple H^+ diffusion channel (not ATP synthase) is inserted into the membrane, the energy of the H^+ gradient is released as heat, rather than being coupled to the synthesis of ATP. Such uncoupling molecules are deliberately used by some organisms to generate heat. For example, uncoupling the protein thermogenin plays an important role in regulating the temperature of some mammals, especially newborn human infants, who lack the hair to keep warm, and of hibernating animals. We will describe this process in more detail in Chapter 40.

Fermentation: ATP from Glucose, without O_2

Suppose the supply of oxygen to a respiring cell is cut off (an anaerobic condition), perhaps by drowning or by extreme exertion. As we can deduce from Figure 7.13, the first consequence of an insufficient supply of O_2 is that the cell cannot reoxidize cytochrome c, so all of that compound is soon in the reduced form. When this happens, QH_2 cannot be oxidized back to Q, and soon all the Q is in the reduced form. So it goes, until the entire respiratory chain is reduced. Under these circumstances, no NAD^+ and no FAD are generated from their reduced forms. Therefore, the oxidative steps in glycolysis, pyruvate oxidation, and the citric acid cycle also stop. If the cell has no other way to obtain energy from its food, it will die.

Under anaerobic conditions, many (but not all) cells can continue to carry out glycolysis and produce a limited amount of ATP by fermentation. This process occurs in the cytoplasm with glycolysis.

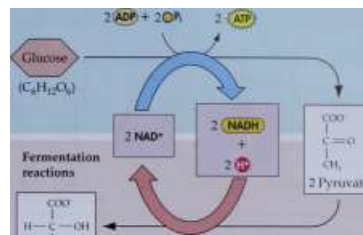
Fermentation has two defining characteristics. First, it uses $NADH + H^+$ formed by glycolysis to reduce pyruvate or one of its metabolites, and consequently NAD^+ is regenerated. NAD^+ is required for reaction 6 of glycolysis (see Figure 7.6), so once the cell has replenished its supply in this way, it can carry more glucose through glycolysis.

Second, fermentation enables glycolysis to produce a small but sustained amount of ATP. Only as much ATP is produced as

can be obtained from substrate-level phosphorylation—not the much greater yield of ATP obtained by cellular respiration.

When cells capable of fermentation become anaerobic, the rate of glycolysis speeds up tenfold or even more. Thus a substantial rate of ATP production is maintained, although

Glycolysis



Fermentation reactions

7.75 Lactic Acid Fermentation

Glycolysis produces pyruvate, as well as ATP and $NADH + H^+$, from glucose. Lactic acid fermentation, using $NADH + H^+$ as the reducing agent, then reduces pyruvate to lactic acid (lactate).

efficiency in terms of ATP molecules per glucose molecule is greatly reduced as compared with aerobic respiration.

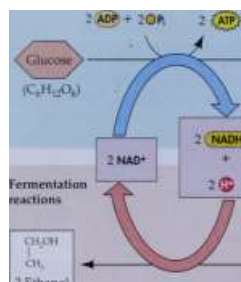
Some organisms are confined to totally anaerobic environments and use only fermentation. Other organisms carry on fermentation even in the presence of oxygen. And several bacteria carry on cellular respiration—not fermentation—without using oxygen gas as an electron acceptor. Instead, to oxidize their cytochromes, these bacteria reduce nitrate ions (NO_3^-) to nitrite ions (NO_2^-). We'll put that observation into a broader context in Chapter 26.

Some fermenting cells produce lactic acid and others produce alcohol

Many different types of fermentation are carried out by different bacteria and eukaryotic body cells. These different fermentations are distinguished by the final product produced. For example, in lactic acid fermentation, pyruvate is reduced to lactate (Figure 7.15). Lactic acid fermentation takes place in many microorganisms as well as in our muscle cells. Unlike muscle cells, however, nerve cells (neurons) are incapable of fermentation because they lack the enzyme that reduces pyruvate to lactate. For this reason, without adequate oxygen, the human nervous system (including the brain) is rapidly destroyed; it is the first part of the body to die.

Certain yeasts and some plant cells carry on a process called alcoholic fermentation under anaerobic conditions (Figure 7.16). This process requires two enzymes to metabolize pyruvate. First, carbon dioxide is removed from pyruvate, leaving the compound acetaldehyde. Second, the acetaldehyde is reduced by $NADH + H^+$, producing NAD^+ and ethyl alcohol (ethanol). The brewing industry relies on alcoholic fermentation to produce wine and beer.

Glycolysis



>

COO"

I

c=O

I

CH₃, 2 Pyruvate

^

co 2 co 2

CHO

I

CH₃

2 Acetaldehyde

7.76 Alcoholic Fermentation

In alcoholic fermentation (the basis for the brewing industry), pyruvate from glycolysis is converted to acetaldehyde and CO₂ is released. The NADH + H⁺ from glycolysis acts as a reducing agent, reducing acetaldehyde to ethanol.

Contrasting Energy Yields

The total net energy yield from fermentation is two molecules of ATP per molecule of glucose oxidized. In contrast, the maximum yield that can be obtained from a molecule of glucose through glycolysis followed by complete aerobic respiration is much greater—about 36 molecules of ATP (Figure 7.17). (You can study Figures 7.6, 7.9, and 7.13 to review where these ATP molecules come from.)

Why is so much more ATP produced by aerobic respiration? Fermentation is an incomplete oxidation of glucose. Much more energy remains in the end products of fermentation, such as lactic acid and ethanol, than in CO₂. In cellular respiration, carriers (mostly NAD⁺) are reduced in pyruvate oxidation and the citric acid cycle, then oxidized by the respiratory chain, with the accompanying production of ATP (three for each NADH + H⁺ and two for each FADH₂) by the chemiosmotic mechanism. In an aerobic environment, an organism capable of this type of metabolism will be at an advantage (in terms of energy availability per glucose molecule) over one limited to fermentation.

The total gross yield of ATP from one molecule of glucose taken through glycolysis and cellular respiration is 38. However, we may subtract two from that gross—for a net yield of 36 ATP—because in some animal cells the inner mitochondrial mem-

brane is impermeable to NADH, and a "toll" of one ATP must be paid for each NADH produced in glycolysis that is shuttled into the mitochondrial matrix.

Metabolic Pathways

Glycolysis and the respiratory pathways do not operate in isolation from the rest of metabolism. Rather, there is an in-

7.7 7 Cellular Respiration Yields More Energy Than Glycolysis Does

Carriers are reduced in pyruvate oxidation and the citric acid cycle, then oxidized by the respiratory chain. These reactions produce ATP via the chemiosmotic mechanism.

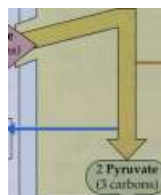
Cytosol

GLYCOLYSIS

/Glucose

v (6 carbons)

! (NADH)



1 (NADH)

Glycolysis yields 2 molecules of ATP for every glucose molecule entering the pathway.



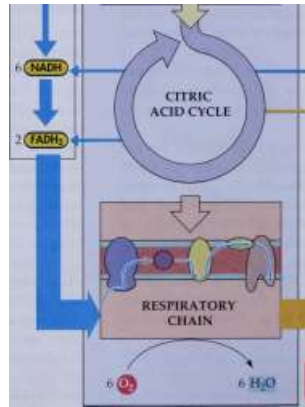
Pyruvate can continue through cellular respiration reactions, or enter fermentation.

FERMENTATION

Mitochondrion

PYRUVATE OXIDATION

2 Acetyl groups as acetyl CoA (2 carbons)



2 Lactate (3 carbons)

or 2 Ethanol (2 carbons) + 2CO₂

■ ► 2CO₂,

■ ► 4 CO₂,

2£ATP.

A

The ensuing citric acid cycle and respiratory chain produce an additional 34 ATP molecules for every glucose molecule. The source of most of these ATP molecules is the oxidation of reduced carriers (produced in glycolysis, pyruvate oxidation, and the citric acid cycle) by the respiratory chain.

1 A V 32 <\$&

Summary of reactants and products: C₆H₁₂O₆ + 6 O₂ → 6CO₂ + 6H₂O + 36

terchange, with biochemical traffic flowing both into these pathways and out of them, to and from the synthesis and breakdown of amino acids, nucleotides, fatty acids, and so forth. Indeed, the energy-harvesting pathways can be thought of, in railway terms, as the "central switching yard," where carbon skeletons enter from other molecules that are broken down to release their energy (catabolism), and carbon skeletons leave to form the major macromolecular constituents of the cell (anabolism). These relationships are summarized in Figure 7.18.

Catabolism and anabolism involve interconversions using carbon skeletons

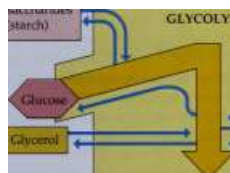
A typical hamburger or vegiburger contains three major sources of carbon skeletons for the person who eats it: carbohydrates, mostly as starch (a polysaccharide); lipids,

CELLULAR RESPIRATION

Lipids (triglycerides)

Polysaccharides (starch)

GLYCOLYSIS



Glycerol

(Pyruvate)

PYRUVATE OXIDATION

W

Fatty acids

Pyrimidines (nucleic acids)

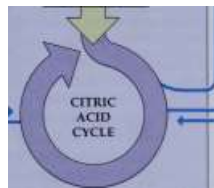
it

Some

amino acids



Acetyl CoA



RESPIRATORY CHAIN

mostly as triglycerides (three fatty acids attached to glycerol); and proteins (polymers of amino acids). Looking at Figure 7.18, you can see how each of these three types of macromolecules can be used in catabolism or anabolism.

catabolic interconversions. Polysaccharides, lipids, and proteins can all be broken down to provide energy.

► Polysaccharides are hydrolyzed to glucose phosphate, an intermediate in glycolysis. This molecule then passes through the rest of glycolysis and the citric acid cycle, where its energy is extracted in NADH and ATP.

► Lipids are converted to their substituents, glycerol and fatty acids. Glycerol is converted to dihydroxyacetone phosphate, an intermediate in glycolysis, and fatty acids to acetate and then acetyl CoA in the mitochondria. In both cases, further oxidation to CO_2 and release of energy then occur.

► Proteins are hydrolyzed to their amino acid building blocks. The 20 amino acids feed into glycolysis or the citric acid cycle at different points. A specific example is shown in Figure 7.19, in which an amino acid is converted to an intermediate in the citric acid cycle.

ANABOLIC INTERCONVERSIONS. As you Can

see in Figure 7.19, many of the pathways for catabolism can operate in reverse. That is, glycolytic and citric acid cycle intermediates, instead of being oxidized to form CO_2 , can be reduced and used to form glucose in a process called gluconeogenesis (which means "new formation of glucose"). Likewise, acetyl CoA can form fatty acids. The most common fatty acids have an even number of carbons: 14, 16, 18, and so forth. These molecules are formed by adding two-carbon acetyl CoA "units" one at a time until the appropriate chain length is reached. Amino acids can be formed by reversing reactions such as the one shown in Figure 7.19, and these amino acids can then be polymerized into proteins.

Some intermediates of the citric acid cycle are used in the synthesis of various important cellular constituents. α -Keto-glutarate is a starting point for purines and oxaloacetate for pyrimidines, both constituents of the nucleic acids DNA and RNA. Succinyl CoA is a starting point for chlorophyll synthesis. Acetyl

Some amino acids ^\

Purines (nucleic acids)

II

Some amino acids

t Proteins

7. 18 Relationships Among the Major Metabolic Pathways of the Cell

Note the central place of glycolysis and the citric acid cycle in this network of metabolic pathways.

132 CHAPTER SEVEN

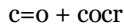
Oxaloacetate is a keto-acid in the citric acid cycle (carbohydrate pathway).

Aspartate is an amino acid (protein pathway).

cocr

CH 2

I'



H-



7.7 9 Coupling Metabolic Pathways

This reaction, in which oxaloacetate (a keto-acid) and aspartate (an amino acid) interconvert, is called a transamination.

CoA is a building block for various pigments, plant growth substances, rubber, and the steroid hormones of animals, among other functions.

Catabolism and anabolism are integrated

A carbon atom from a protein in your burger can end up in DNA or fat or CO_2 , among other fates. How does the cell "decide" which metabolic pathway to follow? With all of the possible interconversions, you might expect that the cellular concentrations of various biochemical molecules would vary widely. For example, the level of oxaloacetate in your cells might depend on what you eat (some food molecules form oxaloacetate) and whether oxaloacetate is used up (in the citric acid cycle or in forming the amino acid aspartate). Remarkably, the levels of these substances in what is called the "metabolic pool" are quite constant. The cell regulates the enzymes of catabolism and anabolism so as to maintain a balance. This metabolic homeostasis gets upset only in unusual circumstances. Let's look at one of them: undernutrition.

Glucose is an excellent source of energy. From Figure 7.18, you can see that fats and proteins are also energy sources. Any one, or all three, can be used to provide the energy you need. In reality, things are not so simple. Proteins, for example, have essential roles in your body as enzymes and structural elements, and using them for energy might deprive you of a catalyst for a vital reaction.

Polysaccharides and fats have no such catalytic roles. But polysaccharides, because they are somewhat polar, can bind a lot of water. Because they are nonpolar, fats do not bind as much water as polysaccharides. So, in water, fats weigh less than polysaccharides. Also, fats are more reduced than carbohydrates (more C—H bonds as opposed to C—OH) and have more energy stored in their bonds. For these two reasons, fats are a better way for an organism to store energy than polysaccharides. It is not surprising that a typical person has about one day's worth of food energy stored as glycogen, a week's food energy as usable proteins food, and over a month's food energy stored as fats.

What happens if a person does not eat enough food to produce sufficient ATP and NADH for anabolism and biological activities? This situation can be the result of a deliberate decision to lose weight, but for too many people, it is

forced upon them because not enough food is available. In either case, the first energy stores in the body to be used are the glycogen stores in muscle and liver cells. This doesn't last long, and next come fats.

The level of acetyl CoA rises as fatty acids are broken down. However, a problem remains: Because fatty acids cannot get from the blood to the brain, the brain can use only glucose as its energy source. With glucose stores already depleted, the body must convert something else to make glucose for the brain. This gluconeogenesis uses mostly amino acids, largely from the breakdown of proteins. So, without sufficient food intake, both proteins (for glucose) and fats (for energy) are used up. After several weeks of starvation, fat stores become depleted, and the only energy source left is proteins, some of which have already been degraded to supply the brain with glucose. At this point, essential proteins, such as antibodies used to fight off infections, get broken down, both for energy and for gluconeogenesis. The loss of these proteins can lead to severe illnesses.

Regulating Energy Pathways

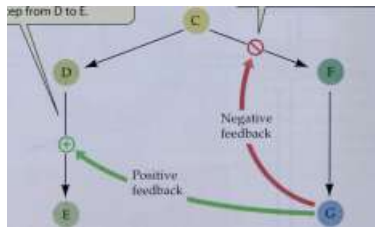
We have described the relationships between metabolic pathways and noted that they work together to provide homeostasis in the cell and organism. But how does the cell regulate these interconversions to maintain constant metabolic pools?

Consider what happens to the starch in your burger bun. In the digestive system, starch is hydrolyzed to glucose, which enters the blood for distribution to the rest of the body. Before this happens, however, a "decision" must be made: Is there already enough glucose in the blood to supply the body's needs? If there is, the excess glucose is con-

Compound G provides positive feedback to the enzyme catalyzing the step from D to E.

;

Compound G inhibits the enzyme for the conversion of C to F, blocking that reaction and ultimately its own synthesis.



7.20 Regulation by Negative and Positive Feedback

Allosteric regulation plays an important role in metabolic pathways. Excess accumulation of some products can shut down their synthesis or stimulate the synthesis of other products.

7.21 Feedback Regulation of Glycolysis and the Citric Acid Cycle

Feedback controls glycolysis and the citric acid cycle at crucial early steps in the pathways, increasing their efficiency and preventing the excessive buildup of intermediates.

GLYCOLYSIS

\Glucose

verted to stored glycogen in the liver. If not enough glucose is supplied by food, liver glycogen is broken down to supply it, or other molecules are used to make glucose by gluconeogenesis.

The end result is that the level of glucose in the blood is remarkably constant. We will describe the details of how this happens in Part Six of this book. For now, it is important to realize that the interconversions of glucose involve many steps, each catalyzed by an enzyme, and it is here that the controls often reside.

Allostery regulates metabolism

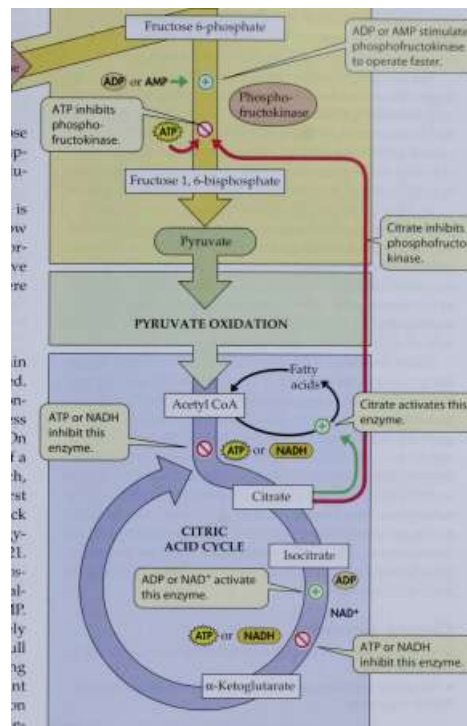
Glycolysis, the citric acid cycle, and the respiratory chain are regulated by allosteric control of the enzymes involved. In metabolic pathways, as we saw in Chapter 6, a high concentration of the products of a later reaction can suppress the action of enzymes that catalyze an earlier reaction. On the other hand, an excess of the product of one branch of a synthetic chain can speed up reactions in another branch, diverting raw materials away from synthesis of the first product (Figure 7.20). These negative and positive feedback control mechanisms are used at many points in the energy-harvesting processes, which are summarized in Figure 7.21.

The main control point in glycolysis is the enzyme phosphofructokinase (reaction 3 in Figure 7.6). This enzyme is allosterically inhibited by ATP and activated by ADP or AMP. As long as fermentation proceeds, yielding a relatively small amount of ATP, phosphofructokinase operates at full efficiency. But when aerobic respiration begins producing ATP 18 times faster than fermentation does, the abundant ATP allosterically inhibits the enzyme, and the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate declines, as does the rate of glucose utilization.

The main control point in the citric acid cycle is the enzyme isocitrate dehydrogenase, which converts isocitrate to α -ketoglutarate (reaction 3 in Figure 7.9). $\text{NADH} + \text{H}^+$ and ATP are feedback inhibitors of this reaction; ADP and NAD^+ are activators (Figure 7.21). If too much ATP is accumulating, or if $\text{NADH} + \text{H}^+$ is being produced faster than it can be used by the respiratory chain, the conversion of isocitrate is slowed, and the citric acid cycle is essentially shut down. A shutdown of the citric acid cycle would cause large amounts of isocitrate and citrate to accumulate, except that the conversion of acetyl CoA to citrate is also slowed by abundant ATP and $\text{NADH} + \text{H}^+$.

However, a certain excess of citrate does accumulate, and this excess acts as an additional negative feedback inhibitor to slow the fructose 6-phosphate reaction early in glycolysis. Consequently, if the citric acid cycle has been slowed down

ADP or AMP stimulate phosphofructokinase to operate faster.



because of abundant ATP (and not because of a lack of oxygen), glycolysis is shut down as well. Both processes resume when the ATP level falls and they are needed again. Allosteric control keeps these processes in balance.

Another control point involves a method for storing excess acetyl CoA. If too much ATP is being made and the citric acid cycle shuts down, the accumulation of citrate switches acetyl CoA to the synthesis of fatty acids for storage. This is one reason why people who eat too much accumulate fat. These fatty acids may be metabolized later to produce more acetyl CoA.

Evolution has led to metabolic efficiency

Allosteric control of the sort illustrated in Figure 7.21 is one of the most impressive examples of the tight organization

134 CHAPTER SEVEN

that can evolve through natural selection when efficient operation is favored in the competition among organisms for limited resources. Each of the feedback controls regulates a part or various parts of the energy-harvesting pathways and keeps them operating in harmony and balance.

Not just the regulatory systems have evolved; the pathways themselves are the products of evolution. Of the energy-harvesting pathways present in cells today, glycolysis and fermentation are the most ancient. These pathways appeared when the planetary environment was strictly anaerobic and all life was prokaryotic. To this day, the enzymes of glycolysis and fermentation are located in the cytoplasm.

Eventually some cells gained the capacity to perform photosynthesis, which added O₂ to the atmosphere, rendering most environments aerobic. Evolution in the aerobic environment led to the appearance of pyruvate oxidation and the citric acid cycle. Elaboration of membranes, especially in eukaryotic cells, allowed the evolution of chemiosmotic mechanisms for coupling electron transport to ATP production, as in oxidative phosphorylation.

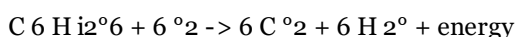
There have also been evolutionary refinements within the pathways themselves. Eukaryotic cells, but not prokaryotic ones, have a cytoskeleton based on microtubules and actin microfilaments (see Figure 4.21). With the appearance of a cytoskeleton, some glycolytic enzymes became attached to cytoskeletal components, which thus organized the enzymes into efficient associations that allow molecules to move from one enzyme in the pathway to the next. In eukaryotes, hexokinase, the first glycolytic enzyme, binds to the outer mitochondrial membrane, giving the enzyme immediate access to ATP produced within the mitochondria. In metabolism, as in all the rest of biology, evolution leads to adaptation.

Chapter Summary

- Metabolic pathways occur in small steps, each catalyzed by a specific enzyme.
- Metabolic pathways are often compartmentalized and are highly regulated.

Obtaining Energy and Electrons from Glucose

- When glucose burns, energy is released as heat and light:



The same equation applies to the metabolism of glucose by cells, but the reaction is accomplished in many separate steps so that the energy can be captured as ATP. Review Figure 7.1

► As a material is oxidized, the electrons it loses are transferred to another material, which is thereby reduced. Such redox reactions transfer large amounts of energy. Much of the energy liberated by the oxidation of the reducing agent is captured in the reduction of the oxidizing agent. Review Figure 7.2

► The coenzyme NAD is a key electron carrier in biological redox reactions. It exists in two forms, one oxidized (NAD^+) and the other reduced ($\text{NADH} + \text{H}^+$). Review Figures 7.3, 7.4

An Overview: Releasing Energy from Glucose

► Glycolysis operates in the presence or absence of O_2 . Under aerobic conditions, cellular respiration continues the breakdown process. Review Figure 7.5

► Pyruvate oxidation and the citric acid cycle produce CO_2 and hydrogen atoms carried by NADH and FADH_2 . The respiratory chain combines these hydrogens with O_2 , releasing enough energy for the synthesis of ATP. Review Figure 7.5

► In some cells under anaerobic conditions, pyruvate can be reduced by NADH to form lactate and regenerate the NAD^+ needed to sustain glycolysis. Review Figure 7.5

► In eukaryotes, glycolysis and fermentation take place in the cytoplasm outside of the mitochondria; pyruvate oxidation, the citric acid cycle, and the respiratory chain operate in association with mitochondria. In prokaryotes, glycolysis, fermentation, and the citric acid cycle take place in the cytoplasm; and pyruvate oxidation and the respiratory chain operate in association with the plasma membrane. Review Table 7.1

Glycolysis: From Glucose to Pyruvate

► Glycolysis is a pathway of ten enzyme-catalyzed reactions located in the cytoplasm. Glycolysis provides starting materials for both cellular respiration and fermentation. Review Figure 7.7

► The energy-investing reactions of glycolysis use two ATPs per glucose molecule and eventually yield two glyceraldehyde 3-phosphate molecules. In the energy-harvesting reactions, two NADH molecules are produced, and four ATP molecules are generated by substrate-level phosphorylation. Two pyruvates are produced for each glucose molecule. Review Figures 7.6, 7.7

Pyruvate Oxidation

► The pyruvate dehydrogenase complex catalyzes three reactions: (1) Pyruvate is oxidized to the acetyl group, releasing one CO_2 molecule and considerable energy; (2) some of this energy is captured when NAD^+ is reduced to $\text{NADH} + \text{H}^+$; and (3) the remaining energy is captured when the acetyl group is combined with coenzyme A, yielding acetyl CoA. Review Figure 7.8

The Citric Acid Cycle

► The energy in acetyl CoA drives the reaction of acetate with oxaloacetate to produce citrate. The citric acid cycle is a series of reactions in which citrate is oxidized and oxaloacetate regenerated (hence a "cycle"). It produces two CO_2 , one FADH_2 , three NADH, and one ATP for each acetyl CoA. Review Figures 7.9, 7.10

The Respiratory Chain: Electrons, Proton Pumping, and ATP

► $\text{NADH} + \text{H}^+$ and FADH_2 from glycolysis, pyruvate oxidation, and the citric acid cycle are oxidized by the respiratory chain, regenerating NAD^+ and FAD. Most of the enzymes and other electron carriers of the chain are part of the inner mitochondrial membrane. Oxygen (O_2) is the final acceptor of electrons and protons, forming water (H_2O). Review Figures 7.11, 7.12

► The chemiosmotic mechanism couples proton transport to oxidative phosphorylation. As the electrons move along the respiratory chain, they lose energy, which is captured by proton pumps that actively transport H^+ out of the mitochondria-

CELLULAR PATHWAYS THAT HARVEST CHEMICAL ENERGY 135

al matrix, establishing a gradient of both proton concentration and electric charge—the proton-motive force. Review Figure 7.13

► The proton-motive force causes protons to diffuse back into the mitochondrial interior through the membrane channel protein ATP synthase, which couples that diffusion to the production of ATP. Several key experiments demonstrate that chemiosmosis produces ATP. Review Figure 7.14

Fermentation: ATP from Glucose, without O_2

► Many organisms and some cells live without O_2 , deriving all their energy from glycolysis and fermentation. Together, these pathways partly oxidize glucose and generate energy-containing products such as lactic acid or ethanol. Fermentation reactions anaerobically oxidize the $\text{NADH} + \text{H}^+$ produced in glycolysis. Review Figures 7.15, 7.16

Contrasting Energy Yields

► For each molecule of glucose used, fermentation yields 2 molecules of ATP. In contrast, glycolysis operating with pyruvate

oxidation, the citric acid cycle, and the respiratory chain yields up to 36 molecules of ATP per molecule of glucose. Review Figure 7.17

Metabolic Pathways

► Catabolic pathways feed into the respiratory pathways. Polysaccharides are broken down into glucose, which enters glycolysis. Glycerol from fats also enters glycolysis, and acetyl CoA from fatty acid degradation enters the citric acid cycle. Proteins enter glycolysis and the citric acid cycle via amino acids. Review Figures 7.18, 7.19

► Anabolic pathways use intermediate components of respiratory metabolism to synthesize fats, amino acids, and other essential building blocks for cellular structure and function. Review Figures 7.18, 7.19

Regulating Energy Pathways

► The rates of glycolysis and the citric acid cycle are increased or decreased by the actions of ATP, ADP, NAD⁺, or NADH + H⁺ on allosteric enzymes.

► Inhibition of the glycolytic enzyme phosphofructokinase by abundant ATP from oxidative phosphorylation slows down glycolysis. ADP activates this enzyme, speeding up glycolysis. The citric acid cycle enzyme isocitrate dehydrogenase is inhibited by ATP and NADH and activated by ADP and NAD⁺. Review Figures 7.20, 7.21

For Discussion

1. Trace the sequence of chemical changes that occurs in mammalian brain tissue when the oxygen supply is cut off. (The first change is that the cytochrome c oxidase system becomes totally reduced, because electrons can still flow from cytochrome c but there is no oxygen to accept electrons from cytochrome c oxidase. What are the remaining steps?)
2. Trace the sequence of chemical changes that occurs in mammalian muscle tissue when the oxygen supply is cut off. (The first change is exactly the same as that in Question 1.)
3. Some cells that use the citric acid cycle and the respiratory chain can also thrive by using fermentation under anaerobic conditions. Given the lower yield of ATP (per molecule of glucose) in fermentation, why can these cells function so efficiently under anaerobic conditions?
4. Describe the mechanisms by which the rates of glycolysis and of aerobic respiration are kept in balance with one another.



Photosynthesis: Energy from the Sun

FOR SEVERAL DECADES, CORN GROWERS IN THE United States competed to see who could coax the highest yield of grain from their acreage. After rising rapidly in the first half of the twentieth century, yields continued to increase, albeit somewhat more slowly. But the trend was clearly up—until the last decade of the century. From 1990 on, crop yields per acre have leveled off for corn, rice, and wheat—three grains which together supply over half the human race with food.

Although overall food production continues to rise as more land is put into production and the environment of the crops is more intensively manipulated with fertilizers and pesticides, the increase of the human population is wiping out any per capita gains. Per person food production has not improved much since 1960-1980, the peak of the so-called "Green Revolution" in agriculture. This was a period when new genetic strains and more intensive environmental management combined to more than double crop yields.

To coax crop plants to grow more and produce more on the available land, scientists are now focusing on photosynthesis, the biochemical process by which plants turn sunlight into carbohydrates, sugars, and starch. Photosynthesis is the very basis of life on Earth.

The basic transformation of photosynthesis—the conversion of solar energy into chemical energy—is a familiar example of the laws of thermodynamics. As the first law tells us, when the form of energy changes from sunlight to plant, no energy is lost. However, as the second law states, the conversion is relatively inefficient, with only about 4 percent of the incident solar energy ending up in chemical bonds. Moreover, the use of solar energy initially captured as ATP and reduced electron carriers to reduce carbon dioxide to sugars is also inefficient.

How can these efficiencies be improved? An important first step is a thorough understanding of photosynthesis. The process of photosynthesis can be neatly broken down into two steps. The first step is the conversion of energy from light to chemical bonds in reduced electron carriers and ATP. In the second step, these two sources of chemical energy are used to drive the synthesis of carbohydrates from carbon dioxide. In this chapter, we will examine these

Primary Producers

Powered by sunlight, corn plants (*Zea mays*) convert atmospheric CO₂ and water into an energy source (food) for humans and animals.

two processes, and show how they are related to each other and to plant growth.

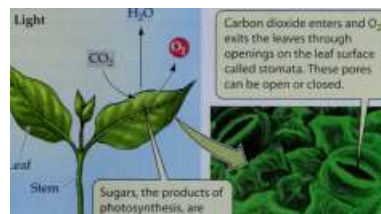
Identifying Photosynthetic Reactants and Products

By the beginning of the nineteenth century, scientists understood the broad outlines of photosynthesis. It was known to use three principal ingredients—water, carbon dioxide (CO_2), and light—and to produce not only carbohydrate but also oxygen gas (O_2). Scientists had learned that:

- ▶ The water for photosynthesis in land plants comes primarily from the soil and must travel from the roots to the leaves.
- ▶ Carbon dioxide is taken in, and water and O_2 are released, through tiny openings in leaves, called stomata (singular stoma) (Figure 8.1)
- ▶ Light is absolutely necessary for the production of oxygen and carbohydrate.



Carbon dioxide enters and O_2 exits the leaves through openings on the leaf surface called stomata. These pores can be open or closed.



Leaf

Sugars, the products of | photosynthesis, are transported throughoi the plant body.



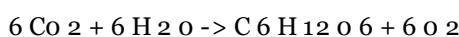
8.1 Ingredients for Photosynthesis

A typical terrestrial plant uses light from the sun, water from the soil, and carbon dioxide from the atmosphere to form organic compounds by photosynthesis.

By 1804, scientists could summarize photosynthesis as follows:

carbon dioxide + water + light energy \rightarrow sugar + oxygen

which turns into an equation that is the reverse of the overall equation for cellular respiration given in Chapter 7:

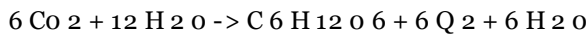


Although correct, these statements say nothing about the details of the process. What roles does light play? How do the carbons become linked? And does the oxygen gas come from the CO_2 or from the H_2O ?

Almost a century and a half passed before the source of the O_2 released during photosynthesis was determined. Its identification was one of the first uses of an isotopic tracer in biological research. In these experiments, two groups of green plants were allowed to carry on photosynthesis. Plants in the first group were supplied with water containing the heavy-

oxygen isotope ^{18}O and with CO_2 , containing only the common oxygen isotope ^{16}O ; plants in the second group were supplied with CO_2 labeled with ^{18}O and water containing only ^{16}O .

When oxygen gas was collected from each group of plants and analyzed, it was found that O_2 containing ^{18}O was produced in abundance by the plants that had been given ^{18}O -labeled water, but not by the plants given labeled CO_2 . These results showed that all the oxygen gas produced during photosynthesis comes from water (Figure 8.2). This discovery is reflected in a revised balanced equation:



Water appears on both sides of the equation because water is both used as a reactant (the twelve molecules on the left) and released as a product (the six new ones on the right). In this revised equation, there are now sufficient water molecules to account for all the oxygen gas produced.

The photosynthetic production of oxygen by green plants is an important source of atmospheric oxygen, which most organisms—including plants themselves—require in order to complete their respiratory chains and obtain the energy for life.

The Two Pathways of Photosynthesis: An Overview

The overall photosynthetic reaction takes place in the chloroplasts of photosynthetic cells, which in most plants are found in the leaves. But photosynthesis does not proceed in a single step. In fact, in all of chemistry, no such complex reaction is accomplished in a single step. Rather, a series of simpler steps is required.

By the middle of the twentieth century, it was clear that photosynthesis consists of many reactions that can be divided into two pathways:

- The first pathway, called the light reactions, is driven by light energy. It produces ATP and a reduced electron carrier ($\text{NADPH} + \text{H}^+$).

- The second pathway, called the Calvin-Benson cycle, does not use light directly. It uses ATP, $\text{NADPH} + \text{H}^+$, and CO_2 to produce sugar.

EXPERIMENT

n

Question: What is the source of the O_2 produced by photosynthesis?

Experiment 1 H_2^{16}O , C^{18}O_2

METHOD

Plants were given isotope-labeled carbon dioxide (C^{18}O_2), and unlabeled water.



Experiment 2

H_2^{18}O , CO_2

Plants were given isotope-labeled water (H_2^{18}O), and unlabeled CO_2 .



RESULTS

The oxygen released was unlabeled.

The oxygen released was labeled.

Conclusion: Water is the source of the O_2 produced by photosynthesis.

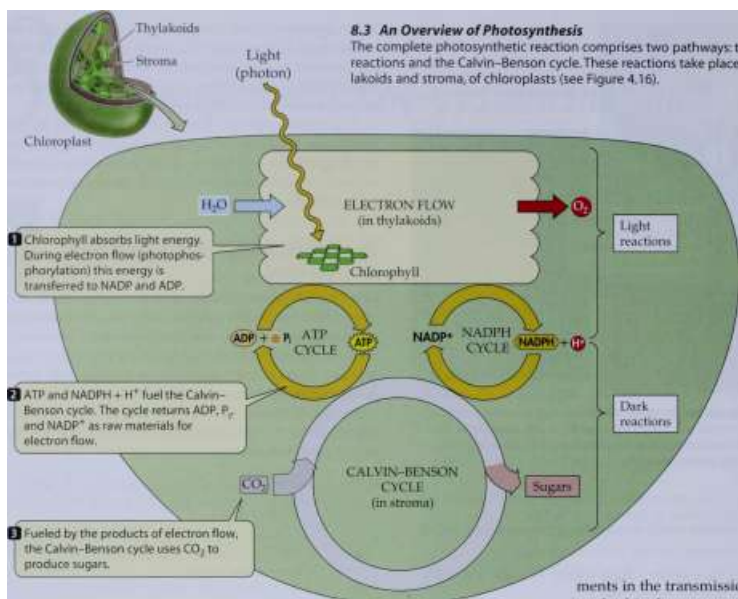
8.2 Water Is the Source of the Oxygen Produced by Photosynthesis

Because only plants given isotope-labeled water released labeled O_2 , this experiment showed that water is the source of the oxygen released during photosynthesis.

138 CHAPTER EIGHT

8.3 An Overview of Photosynthesis

The complete photosynthetic reaction comprises two pathways: the light reactions and the Calvin-Benson cycle. These reactions take place in the thylakoids and stroma, of chloroplasts (see Figure 4.16).



Fueled by the products of electron flow, the Calvin-Benson cycle uses CO_2 to produce sugars.

In the first pathway of photosynthesis—the light reactions — light energy is captured by pigment molecules and used to produce ATP from ADP and P_i . The light reactions are mediated by molecular assemblies called photosystems. These systems pass electrons from one molecule to another, and some of this electron flow is coupled to ATP synthesis. Because light is the ultimate energy source, the synthesis of ATP in this pathway is called photophosphorylation.

The NADPH + H^+ and ATP produced by the light reactions are used in the second pathway, the Calvin-Benson cycle, whose reactions trap CO_2 and reduce the resulting acid to sugar. This pathway is also known as the photosynthetic carbon reduction cycle, or simply the dark reactions (because none of its reactions uses light directly).

The reactions of both pathways proceed within the chloroplast, but they reside in different parts of that organelle (Figure 8.3). Both pathways stop in the dark because ATP synthesis and NADP⁺ reduction require light. The rate of each set of reactions depends on the rate of the other. They are linked by the exchange of ATP and ADP, and of NADP⁺ and NADPH.

Properties of Light and Pigments

Light is a source of both energy and information. In later chapters, we'll examine the many roles of light and pig-

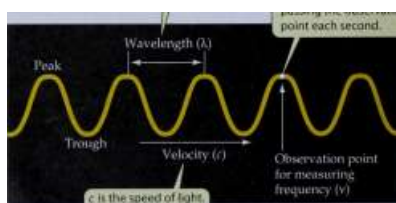
ments in the transmission of information. In this chapter, our focus is on light as a source of energy. We start by examining the physical nature of light.

Light comes in packets called photons

Light is a form of electromagnetic radiation. It comes in discrete packets called photons. Light also behaves as if it were propagated in waves. The wavelength of light is the distance from the peak of one wave to the peak of the next (Figure 8.4). Light and other forms of electromagnetic radi-

λ is the distance between peaks. f

v is the number of peaks passing the observation point each second.



8.4 Light Has Wavelike Properties

Light can be envisioned as a series of waves whose peaks pass a fixed observation point with uniform frequency.

ation—cosmic rays, gamma rays, X rays, ultraviolet radiation, infrared radiation, microwaves, and radio waves— can be classified according to their wavelengths. Visible light fits into this electromagnetic spectrum between ultraviolet and infrared radiation (Figure 8.5).

Humans perceive light as having distinct colors. The colors relate to the wavelengths of the light, as shown in Figure 8.5. Most people can see electromagnetic radiation in the range of wavelengths from 400 to 700 nm. The wavelength at 400 nm marks the violet end of the visible spectrum; the one at 700 nm marks the red end. Wavelengths in the range from about 100 to 400 nm are ultraviolet radiation; those immediately above 700 nm are referred to as infrared.

The speed of light in a vacuum is one of the universal constants of nature. In a vacuum, light travels at 3×10^{10} centimeters per second (or 186,000 miles per second), a value symbolized as c . In air, glass, water, and other media, light travels slightly more slowly.

Let's consider light as a long train of waves moving in a straight line and see what the train would look like to a stationary observer. Successive peaks of the waves pass the observer with a uniform frequency determined by the wavelength and the speed of light. The exact relationship is

$$v = c/\lambda$$

where v (the Greek letter nu) is the frequency; c is the speed of light; and λ (Greek lambda) is the wavelength. Often v is expressed in hertz (Hz), c in centimeters per second (cm/s), and λ in nanometers (nm) ($1 \text{ nm} = 10^{-9} \text{ m}$ or 10^{-7} cm).

The amount of energy, E , contained in a single photon is directly proportional to its frequency. The constant of proportionality that describes this relationship, h , is named Planck's constant, after Max Planck, who first introduced the concept of the photon. With this information we can write the equation

$$E = h \cdot v$$

where v is the frequency in Hz.

Substituting c/λ for v (from the equation above relating λ , v , and c), we see that

$$E = hc/\lambda$$

Thus shorter wavelengths mean greater energies; that is, energy is inversely proportional to wavelength. A photon of red light of wavelength 660 nm has less energy than a photon of blue light of 430 nm; an ultraviolet photon of 284 nm is much more energetic than either of these. For a photon to be active in any light-driven biological process—such as photosynthesis—it must have enough energy to perform the work required.

The brightness, or intensity, of light at a given point is the amount of energy falling on a defined area—such as 1 cm^2 —per second. Light intensity is usually expressed in energy units (such as calories) per square centimeter per second, but the intensity of pure light of a single wavelength may also be expressed in terms of photons per square centimeter per second.

Cosmic rays 8.5 The Electromagnetic Spectrum

Gamma rays y ne portion of the electromagnetic spectrum

I that is visible to humans is shown in detail at

the right. Wavelength (nm) l r AAAAAAAAAAAAAAAAAA



10

Ultraviolet (UV) in 2

Visible light

10-

1(T

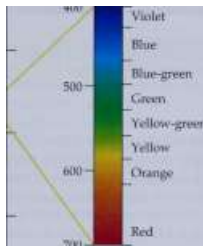
Infrared (IR)

10 s

10 e

Shorter wavelengths are more energetic.

400



700

Longer wavelengths are less energetic.

Microwaves Radio waves

Absorption of a photon puts a pigment in an excited state

When a photon meets a molecule, one of three things happens:

- ▶ The photon may bounce off the molecule—it may be reflected.
- ▶ The photon may pass through the molecule—it may be transmitted.

Neither of these outcomes causes any change in the molecule, and neither has any chemical consequences.

- ▶ The photon may be absorbed by the molecule.

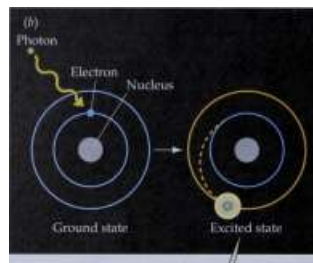
In this case, the photon disappears. Its energy, however, cannot disappear, because energy is neither created nor destroyed.

When a molecule absorbs a photon, it acquires the energy of that photon. It is thereby raised from a ground state (lower energy) to an excited state (higher energy) (Figure

140 CHAPTER EIGHT

/L

When a molecule in the ground state absorbs a photon, it is raised to an excited state and possesses more energy.



The absorption of the photon boosts one of the molecule's electrons to an orbital farther from the nucleus.

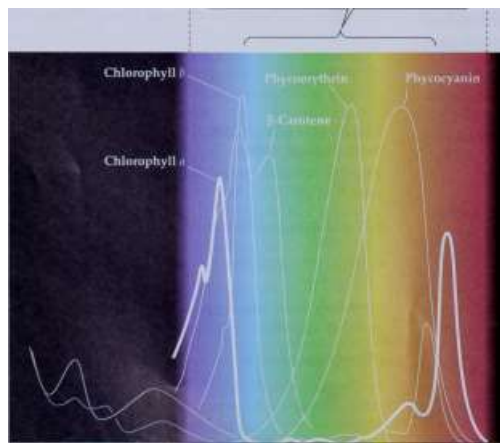
8.6 Exciting a Molecule

(a) When a molecule absorbs the energy of a photon, it is raised from a ground state to an excited state, (b) In the excited state, one of the molecule's electrons is boosted to a higher orbital, where it is held less firmly by the molecule.

8.6(7). The difference in energy between the excited state and the ground state is precisely equal to the energy of the absorbed photon. The increase in energy boosts one of the electrons in the molecule into an orbital farther from the nucleus; this electron is now held less firmly by the molecule (Figure 8.6b), with chemical consequences that we will discuss later in this chapter.

Visible spectrum

Notice how much of the visible spectrum would go to waste if chlorophyll a were the only pigment absorbing light for photosynthesis.



250

All molecules absorb electromagnetic radiation. The specific wavelengths absorbed by a particular molecule are characteristic of that type of molecule. Molecules that absorb wavelengths in the visible region of the spectrum are called pigments.

When a beam of white light (light containing visible light of all wavelengths) falls on a pigment, certain wavelengths of the light are absorbed. The remaining wavelengths, which are reflected or transmitted, make the pigment appear to us to be colored. For example, if a pigment absorbs both blue and red light—as chlorophyll does— what we see is the remaining light— primarily green.

Light absorption and biological activity vary with wavelength

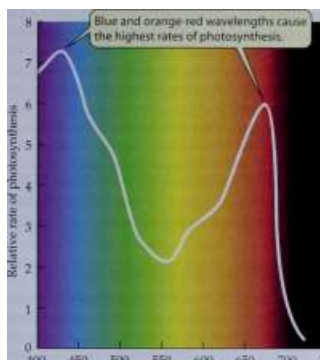
A given type of molecule can absorb radiant energy of only certain wavelengths. If we plot a compound's absorption of light as a function of the wavelengths of the light, the result is an absorption spectrum (Figure 8.7). Absorption spectra are good "fingerprints" of compounds; sometimes an absorption spectrum contains enough information to enable us to identify an unknown compound.

Light can also be analyzed for the magnitude of its effect on a particular

8.7 Photosynthetic Pigments Have Distinct Absorption Spectra

Photosynthesis uses most of the visible spectrum because the participating pigments absorb photons most strongly at different wavelengths.

700



500 550 600 650 Wavelength (nm)

750

8.8 Action Spectrum of Photosynthesis

An action spectrum plots the biological effectiveness of different wavelengths of radiation. Here the rate of photosynthesis in the freshwater plant *Anacharis* is plotted against wavelengths of visible light. If we compare this action spectrum with the absorption spectra of specific pigments, such as those in Figure 8.7, we can identify which pigments are responsible for the process in *Anacharis*.

activity, such as photosynthesis. A plot of the effectiveness of light as a function of wavelength is called an action spectrum. Figure 8.8 shows the action spectrum for photosynthesis by *Anacharis*, a freshwater plant. All wavelengths of visible light are at least somewhat effective in causing photosynthesis, but the blue and orange-red wavelengths are the most effective. Action spectra are helpful in determining what pigment or pigments are being used in a particular photobiological process, such as photosynthesis. We should be able to find which pigment or pigments have absorption spectra that match the action spectrum of the process we are observing.

Photosynthesis uses chlorophylls and accessory pigments

Certain pigments are important in biological processes, and we will discuss them as they appear in this book. Here we discuss the pigments that play roles in photosynthesis. Of these, the most important ones are the chlorophylls. Chlorophylls occur universally in the plant kingdom, in photosynthetic protists, and in photosynthetic bacteria. A mutant individual that lacks chlorophyll is unable to perform photosynthesis and will starve to death.

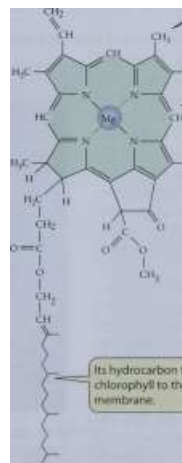
In plants, two chlorophylls predominate: chlorophyll a and chlorophyll b. These two molecules differ only slightly in their molecular structure. Both have a complex ring

structure similar to the heme group of hemoglobin. In the center of each chlorophyll ring is a magnesium atom, and at a peripheral location on the ring is attached a long hydrocarbon "tail" that can adhere the chlorophyll to the hydrophobic portion of the thylakoid membrane (Figure 8.9).

We saw in Figures 8.7 and 8.8 that the chlorophylls absorb blue and red wavelengths, which are near the two ends of the visible spectrum. Thus, if only chlorophyll pigments were active in photosynthesis, much of the visible spectrum would go unused. However, all photosynthetic organisms possess accessory pigments, which absorb photons intermediate in energy between the red and the blue wavelengths, then transfer a portion of that energy to the chlorophylls.

Among these accessory pigments are carotenoids, such as P-carotene (see Figure 3.23), which absorb photons in the blue and blue-green wavelengths and appear deep yellow. The phycobilins (phycoerythrin and phycocyanin), which are found in red algae and in cyanobacteria (contributing to their respective colors), absorb various yellow-green, yellow, and orange wavelengths. Such accessory pigments, in collaboration with the chlorophylls, constitute an energy-absorbing antenna system covering much of the visible spectrum.

Chlorophyll a



In chlorophyll b, this methyl group is replaced by an aldehyde group, —CHO.

ch,

/

J Light is absorbed by the

g structure of

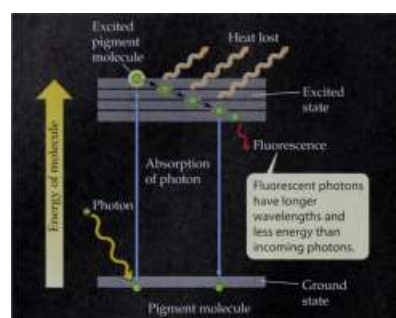
I complex rin I chlorophyll.

CH,

Its hydrocarbon tail secures chlorophyll to the thylakoid membrane.

8.9 The Molecular Structure of Chlorophyll

Chlorophyll consists of a complex ring with a magnesium atom (shaded area) at the center, plus a hydrocarbon "tail."



An excited pigment molecule may give off some of its absorbed energy as fluorescent light when the molecule returns to its ground state.

Light Reactions: Light Absorption

A pigment molecule enters an excited state when it absorbs a photon (see Figure 8.6). The excited state is an unstable potential energy state, and the molecule usually does not stay in it very long. One of two things happens:

- The molecule returns to the ground state, emitting much of the absorbed energy as fluorescence.
- The molecule passes some of the absorbed energy to another pigment molecule.

In fluorescence, the boosted electron falls back from its higher orbital to its original, lower one (Figure 8.10). This process is accompanied by a loss of energy, which is given off as another photon. The energy of this photon, however, is somewhat less than the energy the pigment absorbed (recall the second law of thermodynamics), and so the emitted photon has a longer wavelength than the absorbed one. In any case, there can be no chemical changes or biological consequences—no chemical work is done.

However, rather than emitting the photon's energy as fluorescence, the pigment molecules may pass the absorbed energy along. The pigments in photosynthetic organisms are arranged into energy-absorbing antenna systems. In these systems, the molecules are held by a complex of proteins in the right orientation for light absorption. Any pigment molecule with a suitable absorption spectrum can absorb an incoming photon and become excited. The excitation passes from one pigment molecule in the antenna to another, moving

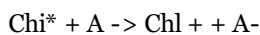
from pigments that absorb shorter wavelengths (higher energies) to pigments that absorb longer wavelengths (lower energies) of light. Thus the excitation ends up in the one pigment molecule in the antenna that absorbs the longest wavelength; this molecule occupies the reaction center of the antenna (Figure 8.11).

The reaction center is the part of the antenna that converts the light absorbed into chemical energy. In plants, the pigment molecule in the reaction center is always a molecule of chlorophyll a. There are many other chlorophyll a molecules in the antenna, but all of them absorb light at shorter wavelengths than does the molecule in the reaction center.

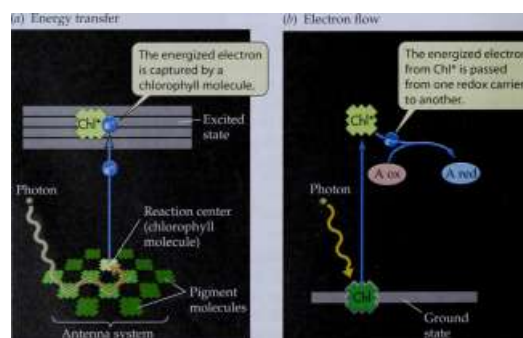
Excited chlorophyll acts as a reducing agent

The light energy absorbed by the antenna system is transferred from one pigment molecule to another as an electron. When this happens, the second molecule is reduced by the first. Recall that reduction is the addition of electrons and oxidation is their removal. Ultimately, however, photosynthesis conserves energy by using the excited chlorophyll molecule in the reaction center as a reducing agent (Figure 8.11).

Ground-state chlorophyll (symbolized as Chi) is not much of a reducing agent, but excited chlorophyll (Chi^*) is a good one. To understand the reducing capability of Chi^* , recall that in an excited molecule, one of the electrons is zipping about in an orbital farther away from its nucleus. Less tightly held, this electron can be passed on in a redox reaction to an oxidizing agent. Thus Chi^* (but not Chi) can react with an oxidizing agent A in a reaction like this:



Electron flow



8.11 Energy Transfer and Electron Flow

Rather than being lost as fluorescence, energy may be transferred from one molecule to another, preserving the energy for biochemical work. (a) An excited molecule can transfer energy to a chlorophyll molecule in the reaction center, (b) In electron flow, the energetic electron from the excited chlorophyll molecule is transferred from one redox carrier to another.

This, then, is the first biochemical consequence of light absorption by chlorophyll: The chlorophyll becomes a reducing agent and participates in a redox reaction that would not have occurred in the dark.

As we are about to see, the further adventures of the electrons from chlorophyll reduce NADP^+ and generate a proton-motive force that is eventually used to synthesize ATP.

Electron Flow, Photophosphorylation and Reductions

The high energy stored in the electrons of excited chlorophyll can be transferred to suitably oxidized nonpigment acceptor molecules. When these molecules are reduced, they can in turn reduce other molecules, setting up an electron flow. Electrons can flow through a series of carriers where the reduced form of one has more energy than the reduced form of the next; this system is similar to electron transport in the mitochondria (Chapter 7), and releases the energy that is captured by the chemiosmotic synthesis of ATP in a process called photophosphorylation. ATP is used

Light

in the dark reactions as a source of energy for the endergonic synthesis of carbohydrate (see Figure 8.3).

A second energy-rich product of the light reactions that is used in the dark reactions is a reduced coenzyme, NADPH + H⁺. Just as NAD⁺ couples the pathways of cellular respiration, a similar compound, NADP⁺ (nicotinamide adenine dinucleotide phosphate) couples the photosynthetic pathways. NADP⁺ is identical to NAD (see Figure 7.3), except that the latter has another phosphate group attached to the ribose. Whereas NAD participates in catabolism, NADP is used in synthetic reactions (anabolism), such as carbohydrate synthesis from CO₂, that require energy through reducing power.

There are two different systems of electron flow in photosynthesis:

- Noncyclic electron flow produces NADPH + H⁺ and ATP.
- Cyclic electron flow produces only ATP.

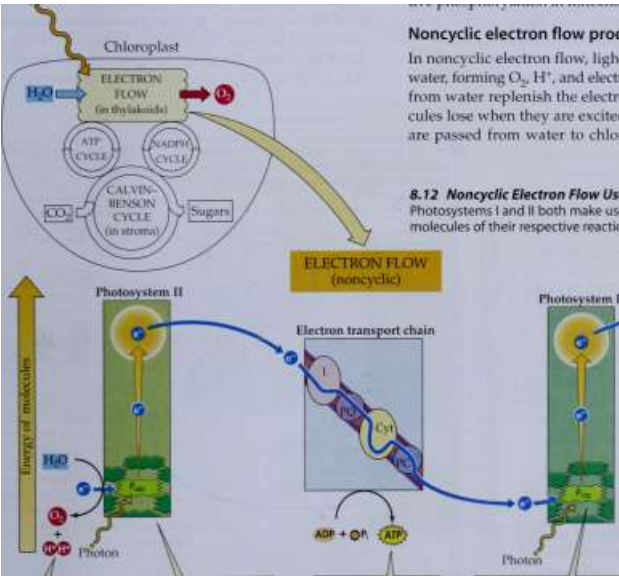
We'll consider these noncyclic and cyclic reactions before considering the role of chemiosmosis in phosphorylation— a process that is very similar to that discovered for oxidative phosphorylation in mitochondria.

Noncyclic electron flow produces ATP and NADPH

In noncyclic electron flow, light energy is used to oxidize water, forming O₂, H⁺, and electrons (Figure 8.12). Electrons from water replenish the electrons that chlorophyll molecules lose when they are excited by light. As the electrons are passed from water to chlorophyll, and ultimately to

8.12 Noncyclic Electron Flow Uses Two Photosystems

Photosystems I and II both make use of the excited chlorophyll molecules of their respective reaction centers.



^*(R)



Fd

NADP+ reductase



NADP+

+

(NADPH) + (H⁺)

Q Photosystem II uses light to oxidize water molecules, producing electrons, H⁺, and O₂.

o The Chl molecule in photosystem II absorbs light maximally at 680 nm, becoming high-energy Chl*.

§| Energy from electron flow through the redox chain is captured for the chemiosmotic synthesis of ATP.

Q The Chl molecule in photosystem I absorbs light maximally at 700 nm, becoming Chl*.

o Photosystem I reduces an oxidizing agent (ferredoxin), which in turn reduces NADP^+ to $\text{NADPH} + \text{H}^+$.

144 CHAPTER EIGHT

NADP^+ , they pass through a series of electron carriers. These redox reactions are exergonic, and some of the free energy released is used ultimately to form ATP by a chemiosmotic mechanism.

Noncyclic electron flow requires the participation of two distinct molecules of chlorophyll. These molecules are associated with two different photosystems, each of which consists of many chlorophyll molecules and accessory pigments in separate energy-absorbing antennas:

- Photosystem I uses light energy to reduce NADP^+ to $\text{NADPH} + \text{H}^+$.
- Photosystem II uses light energy to oxidize water molecules, producing electrons, protons (H^+), and O_2 .

The reaction center for photosystem I contains a chlorophyll molecule in a form called P 700 because it can best absorb light of wavelength 700 nm. The reaction center for photosystem II contains a chlorophyll molecule in a form called P 680 because it absorbs light maximally at 680 nm. Thus photosystem II requires photons that are somewhat more energetic (i.e., lower wavelengths) than those required by photosystem I. To keep noncyclic electron flow going, both photosystems I and II must constantly be absorbing light, thereby boosting electrons to higher orbitals from which they may be captured by specific oxidizing agents.

The reactions of noncyclic electron flow from water to NADP^+ are depicted in Figure 8.12. Photosystem II absorbs photons, sending electrons from P 680 to pheophytin-I—the first carrier in the redox chain—and causing P 680 to become oxidized to P 680^+ . Electrons from

each absorbed by photosystems I and II), one molecule each of NADP^+ and ADP, and one P^- . From these ingredients it produces one molecule each of $\text{NADPH} + \text{H}^+$ and ATP, and half a molecule of oxygen ($\frac{1}{2} \text{O}_2$). A substantial fraction of the light energy absorbed in noncyclic electron flow is lost as heat, but another significant fraction is trapped in ATP and $\text{NADPH} + \text{H}^+$.

Cyclic electron flow produces ATP but no NADPH

Noncyclic electron flow produces equal quantities of ATP and $\text{NADPH} + \text{H}^+$. However, as we will see, the Calvin-Benson cycle uses more ATP than $\text{NADPH} + \text{H}^+$. In order to keep things in balance, plants sometimes make use of a supplementary form of electron flow that does not generate $\text{NADPH} + \text{H}^+$.

Electron flow that produces only ATP is called cyclic because an electron passed from an excited chlorophyll molecule at the outset cycles back to the same chlorophyll molecule at the end of the chain of reactions (Figure 8.13). Water, which supplies electrons to restore chlorophyll molecules to the ground state in noncyclic electron flow, does not enter these reactions; thus they produce no O_2 .

Before cyclic flow begins, P 700, the reaction center chlorophyll of photosystem I, is in the ground state. It absorbs a photon and becomes P 700^* . The P 700^* then reacts with oxidized ferredoxin (Fd_{ox}) to produce reduced ferredoxin (Fd_{red}). The reaction is exergonic, releasing free energy.

the oxidation of water are passed to P 680^+ , reducing it once again to P 680, which can absorb more photons. The electron from photosystem II passes through a series of exergonic reactions in the redox chain, which are coupled to proton pumping. This pumping creates a proton gradient that stores energy for ATP synthesis.

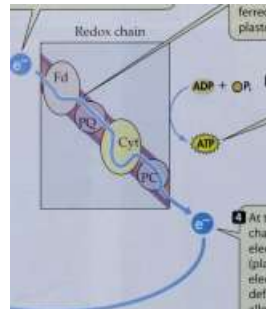
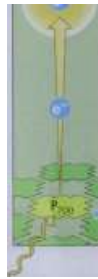
In photosystem I, P 700 absorbs photons, becoming excited to P 700^* , which then leads to the reduction of an oxidizing agent, ferredoxin (Fd), while being oxidized to P 700^+ . Then P 700^+ returns to the ground state by accepting electrons passed through the redox chain from photosystem II. Now electron flow in photosystem II is accounted for, and we must consider only the electrons from photosystem I. These electrons are used in the last step of noncyclic electron flow, in which two electrons and two protons are used to reduce a molecule of NADP^+ to $\text{NADPH} + \text{H}^+$.

In sum, noncyclic electron flow uses a molecule of water, four photons (two

Q In cyclic electron flow, excited electron transport and chlorophylls pass electrons to an oxidizing agent, ferredoxin, leaving positively charged chlorophyll (Chl^+).

/\

Photosystem I



§J Reduced ferredoxin then reduces plastoquinone, and so forth, down the redox chain from ferredoxin through plastocyanin.

§J Energy from electron flow in the redox chain is captured for chemiosmotic synthesis of ATP.

I At the end of the redox chain, the last reduced electron carrier (plastocyanin) passes electrons to electron-deficient chlorophyll, allowing the reactions to start again.

Photon

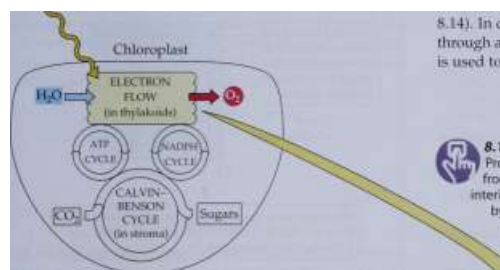
8.73 Cyclic Electron Flow Traps Light Energy as ATP

Cyclic electron flow produces ATP but no $\text{NADPH} + \text{H}^+$. The same chlorophyll molecule passes on the electrons that start the reactions and receives the electrons at the end to start the process over again.

In noncyclic electron flow, Fd red reduces NADP^+ to form $\text{NADPH} + \text{H}^+$. However, 7d Kd can also pass its added electron to a different oxidizing agent, plastoquinone (PQ, a small organic molecule). This is what happens in cyclic flow, which occurs in some organisms when the ratio of $\text{NADPH} + \text{H}^+$ to NADP^+ in the chloroplast is high.

Thus, Fd red reduces PQ, and PQ red passes the electron to a cytochrome complex (Cyt). The electron continues down the redox chain until it completes its cycle by returning to $\text{P}700$. This cycle is a series of redox reactions, each exergonic, and the released energy is stored in a form that ultimately can be used to produce ATP.

Light



When $\text{P}700^+$ passed its electron on to Fd, it became positively charged $\text{P}700^+$. In due course, $\text{P}700^+$ interacts with the last reducing agent in the redox chain, plastocyanin (PC), which donates an electron to $\text{P}700^+$, resulting in a restoration of its uncharged form. By the time the electron from $\text{P}700^+$ travels through the redox chain and comes back to reduce $\text{P}700^+$, all the energy from the original photon has been released. In each of the redox reactions, some free energy is used to form ATP and some free energy is lost as heat.

Chemiosmosis is the source of ATP

In Chapter 7 we considered the chemiosmotic mechanism for ATP formation in the mitochondrion. The chemiosmotic mechanism also operates in photophosphorylation (Figure 8.14). In chloroplasts, as in mitochondria, electrons move through a series of redox reactions, releasing energy, which is used to transport protons (H^+) across a membrane. This

8.14 Chloroplasts Form ATP Chemiosmotically

Protons (H^+) pumped across the thylakoid membrane from the stroma during photophosphorylation make the interior of the thylakoid more acidic than the stroma. Driven by this pH difference, the protons diffuse back to the stroma through ATP synthase channels, which couple the energy of proton flow to the formation of ATP from $\text{ADP} + \text{P}_i$.

Electron transport

r

J^.

Thylakoid interior

High concentration of H (low pH)

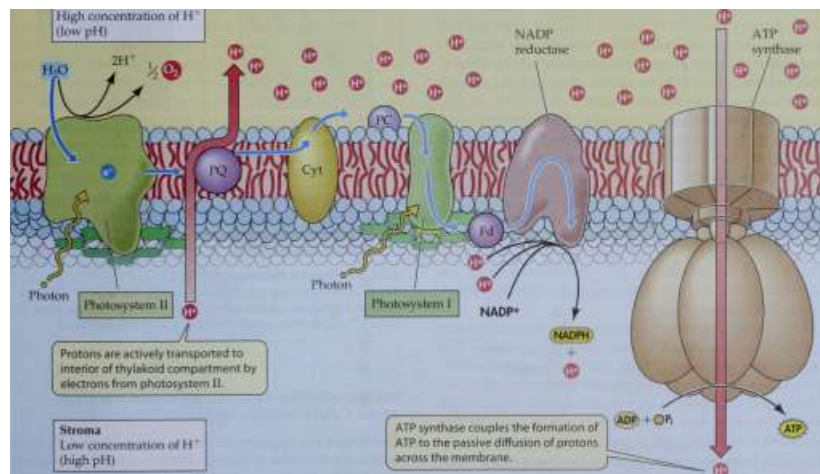
ATP synthesis

^ r

a

o Photon

Photosystem II



Protons are actively transported to interior of thylakoid compartment by electrons from photosystem II.

Stroma

Low concentration of H

(high pH)

ATP synthase couples the formation of ATP to the passive diffusion of protons across the membrane.

146 CHAPTER EIGHT

active proton transport results in a proton-motive force—a difference in pH and in electric charge across the membrane.

In the mitochondrion, protons are pumped out of the matrix, across the inner membrane, and into the space between the inner and outer mitochondrial membranes (see Figure 7.13). Similarly, in the chloroplast, the electron carriers in the thylakoid membranes are oriented so that protons move into the interior of the thylakoid, and the inside becomes acidic with respect to the outside. The ratio of H^+ inside versus outside a thylakoid is usually 10,000:1, which is a difference of 4 pH units. This difference in pH leads to the diffusion of H^+ back out of the thylakoid through specific protein channels in the membrane. These channels are enzymes—ATP synthases—that couple the formation of ATP to the diffusion of protons back across the membrane, just as in mitochondria.

Photosynthetic pathways are the products of evolution

The first photosynthetic organisms were probably anaerobic bacteria that used hydrogen sulfide, rather than water, as a source of electrons:



Many bacteria still use this system, which releases sulfur rather than oxygen.

Nearly 3 billion years ago, the evolution of new pigments in certain bacteria allowed them to extract electrons from water and use them to reduce NADP⁺ while producing O_2 as a by-product. At this time, the atmosphere of Earth contained little O_2 . Over hundreds of millions of years, these cyanobacteria poured enough oxygen gas into the atmosphere to make possible the evolution of cellular respiration. These new photosynthetic reactions forever changed the Earth and the course of evolution, making possible a great diversification of life.

Furthermore, if a larger cell engulfed one of these cyanobacteria, that cell could perform photosynthesis. This was probably

the first event in the evolution of eukaryotic, photosynthetic plant cells.

Making Sugar from CO_2 : The Calvin-Benson Cycle

The second main pathway of photosynthesis is the Calvin-Benson cycle. The reactions of this pathway incorporate CO_2 into sugars.

Most of the enzymes that catalyze the reactions of this pathway are dissolved in the chloroplast stroma (the "soup" outside the thylakoids), and this is where the reactions take place. These reactions are sometimes called the "dark reactions" because they do not directly require light energy. However, they use the energy in ATP and NADPH, produced in the thylakoids during the light reactions, to reduce CO_2 to carbohydrate. So these reactions require light indirectly, and they take place only in the light.

EXPERIMENT

Question: What is the pathway of CO_2 fixation in photosynthesis?

METHOD

Bright light source (energy for photosynthesis)

CO_2 was injected here

Thin flask of green algae



Algae were rapidly killed and their metabolites partially extracted by putting the cells in boiling ethanol.

The plant extract was spotted here and run in two directions to separate compounds from one another.

Paper chromatogram -

UV light

}

First run

Second run

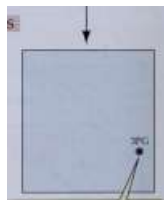
After separation, the chromatogram was overlaid with X-ray film that the radiation "exposed." Each dark spot is a compound labeled with ^{14}C .

\

RESULTS

A chromatogram made after 30 seconds of exposure to $^{14}\text{CO}_2$ shows ^{14}C in many molecules.

Conclusion: The carbon from CO_2 ends up in many molecules.



A chromatogram made after 3 seconds of exposure to $^{14}\text{CO}_2$ shows ^{14}C only in 3PG.

Conclusion: The initial product of CO_2 fixation is 3PG.

8.15 Tracing the Pathway of CO_2

The historical photograph at the top shows the apparatus Calvin and his colleagues used to follow labeled carbon dioxide molecules ($^{14}\text{CO}_2$) as they were transformed by photosynthesis.

Isotope labeling experiments reveal the steps of the Calvin-Benson cycle

To identify the sequence of reactions by which CO_2 ends up in carbohydrate, it was necessary to label CO_2 so that it could be followed after presentation to a plant cell. In the

1950s, Melvin Calvin, Andrew Benson, and their colleagues used radioactively labeled CO_2 in which some of the carbon atoms were not the normal ^{12}C , but its radioisotope ^{14}C . Although ^{14}C is distinguished by its emission of radiation, chemically it behaves virtually identically to nonradioactive ^{12}C . In general, enzymes do not distinguish between isotopes of an element in their substrates, so $^{14}\text{CO}_2$ is treated the same way by photosynthesizing cells as $^{12}\text{CO}_2$.

Calvin and his colleagues exposed cultures of the unicellular green alga *Chlorella* to $^{14}\text{CO}_2$ for 30 seconds. They killed the cells, extracted their carbohydrates, and separated the different compounds from one another by paper chromatography. Many compounds, including monosaccharides and amino acids, contained ^{14}C (Figure 8.15). However, if they stopped the exposure after just 3 seconds, only one compound was labeled—a three-carbon sugar phosphate called 3-phosphoglycerate (3PG):

©OO Carboxyl group

H—@—OH

H—@—O— O

H 3-Phosphoglycerate (3PG)

By tracing the steps in this manner, they soon discovered a cycle, similar to the citric acid cycle, that "fixes" CO_2 in a larger molecule, produces carbohydrate, and regenerates the initial CO_2 acceptor. This cycle was appropriately named the Calvin-Benson cycle.

The initial reaction in the Calvin-Benson cycle fixes the one-carbon CO_2 in a five-carbon compound, ribulose 1,5-bisphosphate (RuBP). An intermediate six-carbon compound forms, which quickly breaks down, forming two three-carbon molecules of 3PG, which is what Calvin and colleagues had seen (Figure 8.16). The enzyme that catalyzes the fixation reaction, ribulose bisphosphate carboxy-

lase/oxygenase (rubisco), is the most abundant protein in the world, comprising about 20 percent of all the protein in every plant leaf.

The Calvin-Benson cycle is composed of three processes

The Calvin-Benson cycle uses the high-energy compounds made in the thylakoids during the light reactions (ATP, NADPH) to reduce CO_2 to carbohydrate. There are three processes that make up the cycle (Figure 8.17):

- Fixation of CO_2 . As we saw, this reaction is catalyzed by rubisco, and its product is 3PG.
- Conversion of fixed CO_2 into carbohydrate (G3P). This series of reactions involves a phosphorylation (using the ATP made in the light reactions) and a reduction (using the NADPH made in the light reactions).
- Regeneration of the CO_2 acceptor, RuBP. Most of the 3PG ends up as RuMP (ribulose monophosphate), and ATP is used to convert this to RuBP. So for every "turn" of the cycle, with one CO_2 fixed, the acceptor gets regenerated.

The end product of this cycle is glyceraldehyde 3-phosphate (G3P), which is a three-carbon sugar phosphate, also called triose phosphate:

H

@=O

H—@—OH

H— <§— O — O

H Glyceraldehyde 3-phosphate (G3P)

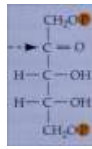
There are two fates for the G3P that ends up as a product of the Calvin-Benson cycle. In a typical leaf, about a third of it ends up in the polysaccharide starch, which is stored in the chloroplast and serves as a source of glucose. Two-thirds of the G3P product is converted to the disaccharide sucrose,

The fate of the carbon atom in CO_2 is followed in red.

--@O₂

Carbon dioxide

| The enzyme rubisco catalyzes the reaction of CO_2 with RuBP.



Ribulose 1,5-bisphosphate (RuBP)

Rubisco

(9

@

®

@

Six-carbon skeleton of reaction intermediate

The reaction intermediate splits into two molecules of 3-phosphoglycerate (3PG).

CH₂O#

COO"

-► HO — C — H + H — C — OH @00~ CH₂ O#

8.16 RuBP Is the Carbon Dioxide Acceptor

CO₂ is added to a five-carbon compound, RuBP. The resulting six-carbon compound immediately splits into two molecules of 3PG.

148 CHAPTER EIGHT Light



®

ELECTRON

FLOW C⁺ (O₂ .

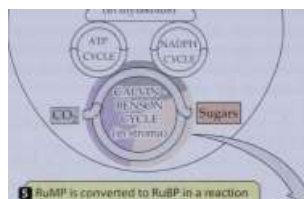
(in thylakoids)



8.17 The Calvin-Benson Cycle

The Calvin-Benson cycle uses CO₂ and the ATP and NADPH + H⁺ generated in the light reactions to produce glucose. This diagram shows only the key steps;

the values given are those necessary to make one molecule of glucose, which requires six "turns" of the cycle.

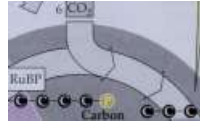
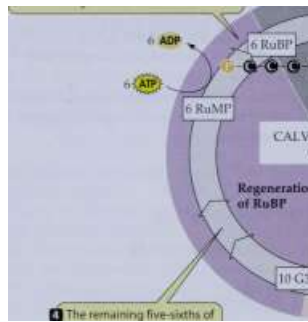


ttMl

Q RuMP is converted to RuBP in a reaction requiring ATP. RuBP is ready to accept another CO₂.

fl CO₂ cor | its acce I forminc

CO₂ combines with its acceptor, RuBP, forming 3PG.



Carbon fixation

CALVIN-BENSON CYCLE

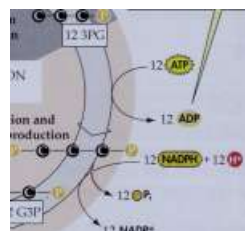
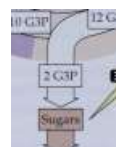
Q3PG is reduced to G3P in a two-step reaction requiring ATP and NADPH + H + .

12 3PG

10G3P

12G3P

| The remaining five-sixths of the G3P is processed in the complex reactions that produce RuMP.



Reduction and sugar production

(NADPH)+12(JP

About one-sixth of the G3P is used to make sugars—the output of the cycle.

Other carbon compounds

which is transported out of the leaf to other organs in the plant, where it is hydrolyzed to its constituent monosaccharides: glucose and fructose.

The glucose produced in photosynthesis is subsequently used by the plant to make other compounds besides sugars. The carbon of glucose is incorporated into amino acids, lipids, and the building blocks of the nucleic acids.

The products of the Calvin-Benson cycle are of crucial importance to the entire biosphere, for the covalent bonds of these products represent the total energy yield from the harvesting of light by plants. Most of this stored energy is released by glycolysis and cellular respiration during plant growth, development, and reproduction. However, much plant matter ends up being consumed by animals. Glycolysis and cellular respiration in the animals releases free energy from the plant matter for use in the animal cells.

Photorespiration and Its Evolutionary Consequences

The properties of rubisco are remarkably identical in all photosynthetic organisms, from bacteria to flowering plants. However, some properties of this enzyme severely limit its effectiveness. In the discussion that follows, we will identify and explore some of these limitations and see how evolution has constructed bypasses around them. First we'll look at photorespiration, a process in which rubisco fixes O_2 instead of CO_2 , lowering the overall rate of CO_2 fixation and plant growth. Then we'll examine some biochemical pathways and features of plant anatomy that compensate for the limitations of rubisco.

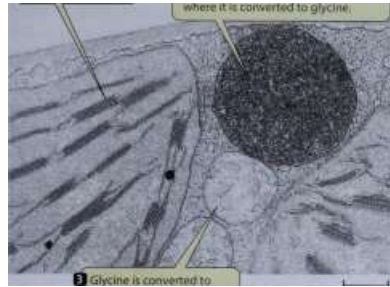
RuBP reacts with O_2 in photorespiration

As its full name indicates, rubisco is a carboxylase (adding CO_2 to an acceptor molecule, RuBP) as well as an oxygenase

Q In the chloroplasts, RuBP reacts with O_2 . Glycolate is formed.

f

Glycolate diffuses into a peroxisome, where it is converted to glycine.



I Glycine is converted to serine in the mitochondria and CO_2 is released.

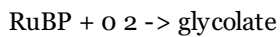
1 |im

8.18 Organelles of Photorespiration

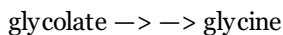
The reactions of photorespiration take place in the chloroplasts, peroxisomes, and, finally, in the mitochondria.

nase (adding O_2 to RuBP). These two reactions compete with each other.

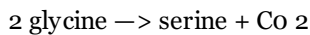
When RuBP and O_2 react, one of the products is a two-carbon compound, glycolate:



The glycolate diffuses into membrane-enclosed organelles called peroxisomes (Figure 8.18). There, a series of reactions converts it to the amino acid glycine:



The glycine then diffuses into a mitochondrion, where two glycine molecules are converted to another amino acid, serine:



This pathway, called photorespiration, uses ATP and NADPH produced in the light reactions, just like the Calvin-Benson cycle. But the net effect of photorespiration essentially undoes what the Calvin-Benson cycle accomplishes: CO_2 is released instead of being fixed into carbohydrate. In many plants, photorespiration reduces the amount of carbon fixed into carbohydrate by 25 percent.

How does rubisco "decide" whether to act as an oxygenase or a carboxylase? The prime consideration is the relative concentrations of CO_2 and O_2 in the leaf. If O_2 is relatively abundant, rubisco acts as an oxygenase, and photorespiration ensues. If CO_2 predominates, rubisco fixes it, and the Calvin-Benson cycle occurs.

The level of O_2 in a leaf becomes especially high on a hot, dry day. To prevent water loss, the stomata that allow water to evaporate from the leaf close (see Figure 8.1). But this also prevents gases from entering and leaving the leaf. So, the CO_2 concentration falls because it is being

PHOTOSYNTHESIS: ENERGY FROM THE SUN 149

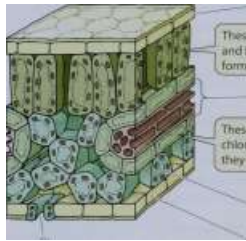
used up by the light-driven photosynthetic reactions, and the O_2 concentration rises because of these same reactions.

If photorespiration is wasteful of the photosynthetic process, why does it exist? Apparently, it is not essential for life. Many plants get along without it, and even plants with it can grow well if it is inhibited chemically. One explanation is that the active site of rubisco evolved to bind both CO_2 and O_2 . This was not a problem originally, as there was little O_2 in the atmosphere, and the CO_2 binding activity was the only one used. When O_2 appeared, so did the photorespiration pathway.

Some plants have evolved systems to bypass photorespiration

A solution to the problem of photorespiration has evolved in a number of plants, most of them related to tropical species such as sugarcane. The objective is to raise the level of CO_2 in relation to O_2 around rubisco, so that carboxylation is favored. This is not an easy task, since the air surrounding a leaf is 21 percent O_2 and only 0.036 percent CO_2 . In the leaves of plants such as roses, wheat, and rice, the mesophyll cells just below the surface are full of chloroplasts that contain abundant rubisco (Figure 8.19a). On a

(a) Arrangement of cells in a C_3 leaf



Upper epidermis

These cells have rubisco and fix CO_2 to form 3PG.

and fix CO_2 to RuBP to

Vein

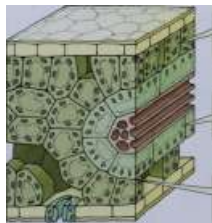
These cells have few chloroplasts and no rubisco; they do not fix CO_2 .

Stoma

(b) Arrangement of cells in a C_4 leaf

Spongy mesophyll cell

Lower epidermis



Mesophyll cells have PEP carboxylase for the reaction of CO_2 and PEP to form a 4-carbon molecule.

Bundle sheath cells have rubisco for the reaction of RuBP with CO_2 released from the 4-carbon compound.

Close association permits CO_2 pumping from mesophyll cells to bundle sheath cells for the Calvin-Benson cycle.

©

8.79 Leaf Anatomy of C_3 and C_4 Plants

Carbon dioxide fixation occurs in different organelles and cells of the leaves in the two types of plants.

150 CHAPTER EIGHT

Q PEP carboxylase in C_4 mesophyll cells catalyzes the formation of the 4-carbon compound oxaloacetate.

hot day, these leaves close their stomata to conserve water. The level of CO_2 in the air spaces of the leaves falls, and that of O_2 rises, as photosynthesis goes on. Because the first product of CO_2 fixation in these plants is the three-carbon molecule 3PG, they are called C_3 plants. As we have seen, photorespiration occurs under these conditions.

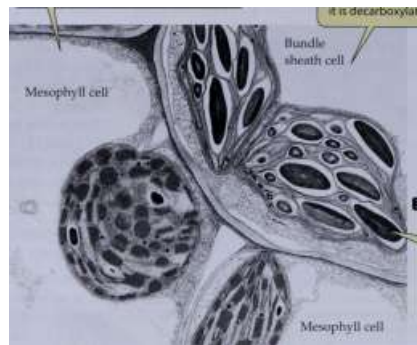
In corn, sugarcane, and other tropical grasses, the chloroplasts, with their abundant rubisco, are in a cell layer in the interior of the leaf (Figure 8.19b). Like C_3 plants, these plants close their stomata on a hot day, but their rate of photosynthesis does not fall, nor does photorespiration occur. They have a way to keep the ratio of CO_2 to O_2 around rubisco high, so that rubisco acts as a carboxylase. They do this in part by having a four-carbon compound, oxaloacetate, as the first product of CO_2 fixation, and so are called C_4 plants.

C₄ plants perform the normal Calvin-Benson cycle, but they have an additional early reaction that fixes CO₂ without losing carbon to photorespiration, greatly increasing the overall photosynthetic yield. Because this initial CO₂ fixation step can function even at low levels of CO₂ and high temperatures, C₄ plants very effectively optimize photosynthesis under conditions that inhibit the photosynthesis of C₃ plants.

C₄ plants have two separate enzymes for CO₂ fixation in different chloroplasts in two different locations in the leaf (Figure 8.20). One, present in the mesophyll cells near the surface of the leaf, fixes CO₂ to a three-carbon acceptor compound (phosphoenolpyruvate) to produce the four-carbon fixation product (oxaloacetate). This enzyme, PEP carboxylase, has two advantages over rubisco:

- It does not have oxygenase activity.
- It fixes CO₂ at very low levels.

So even on a hot day when the stomata are closed, CO₂ is low, and O₂ is high, PEP carboxylase just keeps on fixing CO₂.



Q Oxaloacetate diffuses through plasmodesmata to a bundle sheath cell, where it is decarboxylated, releasing CO₂.

Q Starch grains in the bundle sheath cell indicate that the Calvin-Benson cycle is active and that glucose (and then starch) is being produced.

8.20 C₄ Photosynthesis

Carbon dioxide is fixed initially in the mesophyll cells, but enters the Calvin-Benson cycle in the bundle sheath cells.

Oxaloacetate diffuses out of the mesophyll cells and into the bundle sheath cells in the interior of the leaf. The chloroplasts in bundle sheath cells contain abundant rubisco. There, the four-carbon oxaloacetate is decarboxylated, losing CO₂ and regenerating the three-carbon acceptor, which diffuses back out to the mesophyll cells. The role of this acceptor is to bind CO₂ from the air in the leaf and carry it to the interior cells, where it is "dropped off" at rubisco. This process essentially pumps up the CO₂ concentration around rubisco, so that it acts as a carboxylase and begins the Calvin-Benson cycle.

Kentucky bluegrass, a C₃ plant, thrives on lawns in April and May. But in the heat of summer, it does not do as well, and crabgrass, a C₄ plant, takes over the lawn. The same is true on a global scale for crops: C₃ plants, such as soybeans, rice, wheat, and barley, have been adapted for human food production in temperate climates, while C₄ plants, such as corn and sugarcane, originated and are grown in the tropics. Table 8.1 compares C₃ and C₄ photosynthesis.

C₃ plants are certainly more ancient than C₄ plants. While C₃ photosynthesis appears to have begun about 3.5 billion years ago, C₄ plants appeared about 12 million years ago. A possible factor in the emergence of the C₄ pathway is the decline in atmospheric CO₂. When dinosaurs ruled the Earth 100 million years ago, the concentration of CO₂ was four times what it is now. As CO₂ levels then declined, the more efficient C₄ plants would have had an advantage over their C₃ counterparts.

CAM plants also use PEP carboxylase

Other plants besides the C₄ species use PEP carboxylase to fix and accumulate CO₂ while their stomata are closed. Such plants include some water-storing plants (called succulents) of the family Crassulaceae, many cacti, pineapples, and several other kinds of flowering plants. These plants conserve water by keeping their stomata closed during the daylight hours, thus minimizing water loss by evaporation. How, then, can they perform photosynthesis? Their trick is to open their stomata at night and store CO₂ by a different mechanism.

The CO₂ metabolism of these plants is called crassulacean acid metabolism, or CAM, after the family of succulents in which it was discovered. CAM is much like the metabolism of C₄ plants in that CO₂ is initially fixed into four-carbon compounds. In CAM plants, however, the processes of initial CO₂ fixation and the Calvin-Benson cycle are separated in time rather than in space (Figure 8.21). And CAM plants lack the specialized cell relationships of C₄ plants.

In CAM plants, CO₂ is fixed initially in mesophyll cells to form the four-carbon compound oxaloacetate, which is converted to malic acid. This fixation occurs during the night, when less water is lost through open stomata. When daylight arrives, the accumulated malic acid is shipped to the chloroplasts, where decarboxylation supplies the CO₂ for operation of the Calvin-Benson cycle, and the light reactions supply the necessary ATP and NADPH + H⁺.

Metabolic Pathways in Plants

Green plants are autotrophs, and can synthesize all the molecules they need from three simple starting materials: CO_2 , H_2O , and NH_4^+ . The latter is needed for amino acids, and comes either from the conversion of nitrogen-containing molecules taken up in soil water by the roots or from the bacterial conversion of N_2 gas.

8.21 C_4 and CAM Plants Separate Two Sets of Reactions Differently

Both plant types use four-carbon compounds whose production is separate from the Calvin-Benson cycle. The separation is spatial in C_4 plants, temporal in CAM plants. Sorghum (C_4)

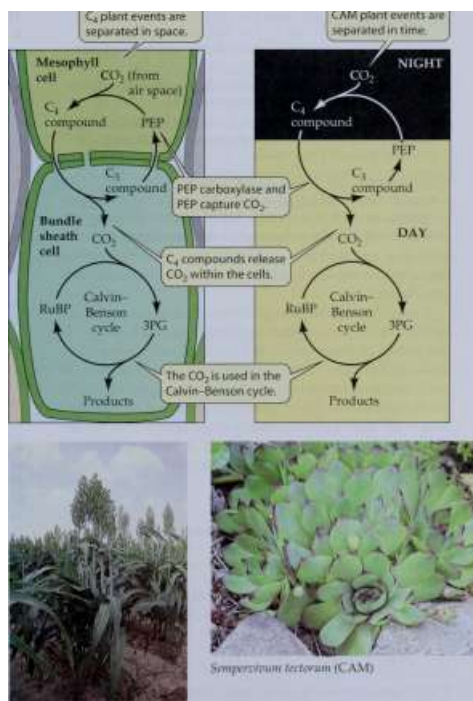
Although the light reactions of photosynthesis generate some ATP and NADPH, the products are used for the Calvin-Benson cycle and are far less than plant cells need to fuel their endergonic reactions. Also, not all plant cells are photosynthetic. To satisfy their need for ATP, plants, like all other organisms, carry out respiration. Both aerobic respiration and fermentation can occur in plants, although the former is far more common.

Plant cellular respiration, unlike photosynthesis, takes place both in the light and in the dark. Because glycolysis occurs in the cytosol, respiration in the mitochondria, and photosynthesis in the chloroplasts, all these processes can proceed simultaneously.

Photosynthesis and respiration are closely linked through the Calvin-Benson cycle (Figure 8.22). Two linkages are particularly important:

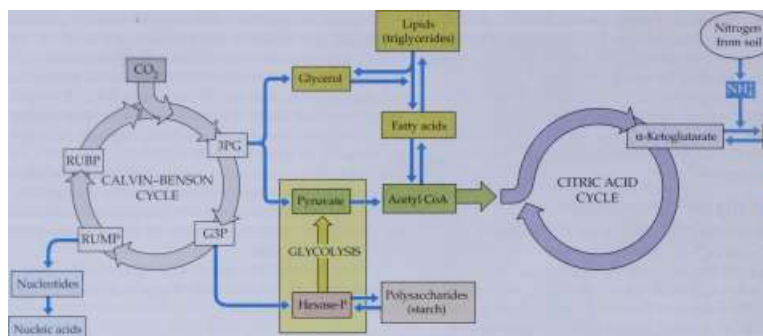
C_4 plant events are separated in space,

CAM plant events are separated in time. 7Z



Sempervivum tectorum (CAM)

152 CHAPTER EIGHT



Amino acids

If

Proteins

8.22 Metabolic Interactions in a Plant Cell

Note the relationships among the Calvin-Benson cycle, the citric acid cycle, and glycolysis.

- ▶ 3PG from the Calvin-Benson cycle can be converted to pyruvate, the end product of glycolysis.
- ▶ G3P from the Calvin-Benson cycle can be converted to hexose phosphates (such as glucose 1-phosphate), which can enter glycolysis.

In both cases, the result is the catabolic breakdown of Calvin-Benson cycle products to CO_2 , with the associated synthesis of ATP. Once the carbon skeletons from the Calvin-Benson cycle enter the "central switching yard" of glycolysis and the citric acid cycle, they can be used anabolically to make lipids, proteins, and other carbohydrates (see Figure 7.19).

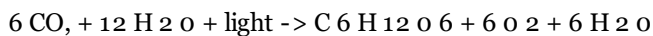
Energy flows from sunlight to reduced carbon in photosynthesis to ATP in respiration. Energy can also be stored in the bonds of macromolecules such as polysaccharides, lipids, and proteins. For a plant to grow, energy storage (as body structures) must exceed energy release; that is, overall photosynthesis to fixed carbon must exceed respiration. This is the aim of the farmers growing corn whom we described in the opening of this chapter. And it is the basis of the ecological food chain, as we will see in later chapters.

Chapter Summary

- ▶ Life on Earth depends on the absorption of light energy from the sun.
- ▶ In plants, photosynthesis takes place in chloroplasts.

Identifying Photosynthetic Reactants and Products

- ▶ Photosynthesizing plants take in CO_2 , water, and light energy, producing O_2 and carbohydrate. The overall reaction is



The oxygen atoms in O_2 come from water, not from CO_2 . Review Figures 8.1, 8.2

The Two Pathways of Photosynthesis: An Overview

- ▶ In the light reactions of photosynthesis, electron flow and photophosphorylation produce ATP and reduce NADP^+ to $\text{NADPH} + \text{H}^+$. Review Figure 8.3
- ▶ ATP and $\text{NADPH} + \text{H}^+$ are needed for the reactions that fix and reduce CO_2 in the Calvin-Benson cycle, forming sugars. Review Figure 8.3

Properties of Light and Pigments

- ▶ Light energy comes in packets called photons, but it also has wavelike properties. Review Figure 8.4
- ▶ Pigments absorb light in the visible spectrum. Review Figure 8.5
- ▶ Absorption of a photon puts a pigment molecule in an excited state that has more energy than its ground state. Review Figure 8.6
- ▶ Each compound has a characteristic absorption spectrum. An action spectrum reveals the biological effectiveness of different wavelengths of light. Review Figures 8.7, 8.8
- ▶ Chlorophylls and accessory pigments form antenna systems for absorption of light energy. Review Figures 8.7, 8.9, 8.11

Light Reactions: Light Absorption

- ▶ An excited pigment molecule may lose its energy by fluorescence, or by transferring it to another pigment molecule. Review Figures 8.10, 8.11

Electron Flow, Photophosphorylation, and Reductions

- ▶ Noncyclic electron flow uses two photosystems (I and II), producing ATP, $\text{NADPH} + \text{H}^+$, and O_2 . Photosystem II uses P 680 chlorophyll, from which light-excited electrons are passed to a redox chain that drives chemiosmotic ATP production. Light-driven oxidation of water releases O_2 and passes electrons from water to the P 680 chlorophyll. Photosystem I passes electrons from P 700 chlorophyll to another redox chain and then to NADP^+ , forming $\text{NADPH} + \text{H}^+$. Review Figure 8.12
- ▶ Cyclic electron flow uses P 700 chlorophyll and produces only ATP. Its operation maintains the proper balance of ATP and $\text{NADPH} + \text{H}^+$ in the chloroplast. Review Figure 8.13
- ▶ Chemiosmosis is the source of ATP in photophosphorylation. Electron transport pumps protons from the stroma into

PHOTOSYNTHESIS: ENERGY FROM THE SUN 153

the thylakoids, establishing a proton-motive force. Diffusion of the protons back to the stroma via ATP synthase channels drives ATP formation from ADP and P_i . Review Figure 8.14

► Photosynthesis probably originated in anaerobic bacteria that used H_2S as a source of electrons instead of H_2O . Oxygen production by bacteria was an important event in the evolution of eukaryotes.

Making Sugar from CO_2 : The Calvin-Benson Cycle

► The Calvin-Benson cycle makes sugar from CO_2 . This pathway was elucidated through the use of radioactive tracers. Review Figure 8.15

► The Calvin-Benson cycle consists of three phases: fixation of CO_2 , reduction and carbohydrate production, and regeneration of RuBP. RuBP is the initial CO_2 acceptor, and 3PG is the first stable product of CO_2 fixation. The enzyme rubisco catalyzes the reaction of CO_2 and RuBP to form 3PG. Review Figures 8.16, 8.17

Photorespiration and Its Evolutionary Consequences

► The enzyme rubisco can catalyze a reaction between O_2 and RuBP in addition to the reaction between CO_2 and RuBP. This consumption of O_2 is called photorespiration and significantly reduces the efficiency of photosynthesis. The reactions that constitute photorespiration are distributed over three organelles: chloroplasts, peroxisomes, and mitochondria. Review Figure 8.18

► At high temperatures and low CO_2 concentrations, the oxygenase function of rubisco is favored.

► C_4 plants bypass photorespiration with special chemical reactions and specialized leaf anatomy. In C_4 plants, PEP carboxylase in mesophyll chloroplasts initially fixes CO_2 in four-carbon acids, which then diffuse into bundle sheath cells, where their decarboxylation produces locally high concentrations of CO_2 . Review Figures 8.19, 8.20

► CAM plants operate much like C_4 plants, but their initial CO_2 fixation by PEP carboxylase is temporally separated from the Calvin-Benson cycle, rather than spatially separated as in C_4 plants. Review Figure 8.21

Metabolic Pathways in Plants

► Plants respire both in the light and in the dark, but photo-synthesize only in the light. To survive, a plant must photo-synthesize more than it respire, giving it a net gain of reduced energy-rich compounds.

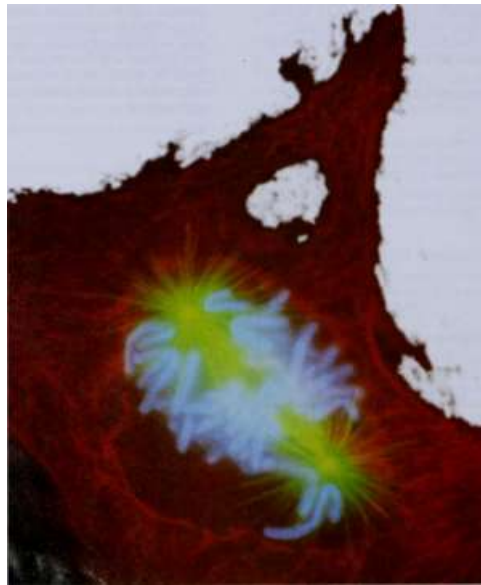
► Photosynthesis and respiration are linked through the Calvin-Benson cycle, the citric acid cycle, and glycolysis. Review Figure 8.22

For Discussion

1. Both electron flow and the Calvin-Benson cycle stop in the dark. Which specific reaction stops first? Which stops next? Continue answering the question "Which stops next?" until you have explained why both pathways have stopped.
2. In what principal ways are the reactions of electron flow in photosynthesis similar to the respiratory chain and oxidative phosphorylation discussed in Chapter 7? Differentiate between cyclic and noncyclic electron flow in terms of (1) the products and (2) the source of electrons for the reduction of oxidized chlorophyll.
3. The development of what two experimental techniques made it possible to elucidate the Calvin-Benson cycle? How were these techniques used in the investigation?
4. If water labeled with ^{18}O is added to a suspension of photo-synthesizing chloroplasts, which of the following compounds will first become labeled with ^{18}O : ATP, NADPH, O_2 , or 3PG? If water labeled with ^3H is added to a suspension of photo-synthesizing chloroplasts, which of the same compounds will first become radioactive? If CO_2 labeled with ^{14}C is added to a suspension of photo-synthesizing chloroplasts, which of those compounds will first become radioactive?

Part Two

Information and Heredity



9

Chromosomes, the Cell Cycle, and Cell Division

■ In 1951, 31-year-old Henrietta Lacks entered Johns Hopkins Hospital to be treated for a

cancerous tumor. Although she died a few months later, her tumor cells are alive today. Scientists found that, given adequate nourishment, cancerous cells from the tumor reproduced themselves indefinitely in a laboratory dish. These "HeLa cells" became a test-tube model for studies of human cell biology and biochemistry. Over the past half-century, tens of thousands of research articles have been published using information obtained from Henrietta's cells. But are these "immortal" cells really a good model for human biology?

In one sense, they are. Most multicellular organisms come from a single cell: the fertilized egg. This cell reproduces itself to make two cells, these in turn divide to become four cells, and so on until all the cells of a new organism have been produced. An organism is not just a ball of many cells, however; the cells must specialize into tissues and organs, each with specific roles to perform. This process of specialization, or differentiation, is a subject we will return to in later chapters of Part Two.

In normal tissues, cell reproduction ("births") is offset by cell loss ("deaths"). We know cell death is important from careful studies of a tiny worm, in which 1,090 cells are produced from the fertilized egg and exactly 131 of them die before the worm is born. If they do not die, the worm's organs are severely malformed. Another example occurs in the mammalian brain. Young mice, for instance, lose hundreds of thousands of brain cells each day; if these cells do not die, the mouse's overcrowded brain simply does not work.

A cell's death is often programmed into its genetic message; normal cells "sacrifice" themselves for the greater good of the organism. Once an organism reaches its adult size, it stays that way through a combination of cell division and programmed cell death. Like most cancerous cells,

Henrietta Lacks's tumor cells, keep growing because they have a genetic imbalance that heavily favors cell reproduction over cell death.

Unicellular organisms use cell division primarily to reproduce themselves, whereas in multicellular organisms cell division also plays important roles in the growth and repair of tissues (Figure 9.1) In this chapter, we first de-

HeLa Cells: More Births Than Deaths

These cells have been cultured in a laboratory since 1951. They are the source of much data relating the reproduction of human cells.

We describe how prokaryotic cells produce two new organisms from the original single-celled organism. Then we describe two types of cell and nuclear division—mitosis and meiosis—and relate these two modes of cell division to asexual and sexual reproduction in eukaryotic organisms. Finally, to balance our discussion of cell "birth" through division, we will describe the important process of programmed cell death, also known as apoptosis.

Systems of Cell Reproduction

In order for any cell to divide, four events must occur:

► There must be a reproductive signal. This signal, which may come either from inside or outside the cell, initiates the cellular reproductive events.

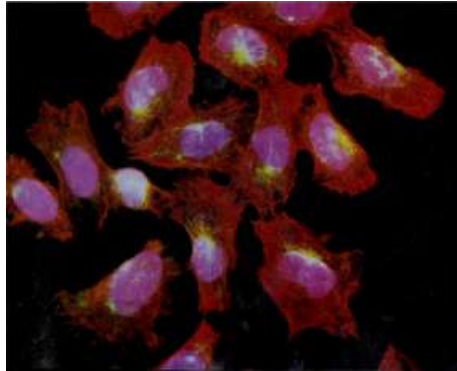
► Replication of DNA, the genetic material, and other vital cell components must occur so that each of the two new cells will have complete cell functions.

► The cell must distribute (segregate) the replicated DNA to each of the two new cells.

► The cell membrane (and the cell wall, in organisms that have one) must grow to separate the two new cells in a process called cytokinesis.

Prokaryotes divide by fission

In prokaryotes, cell division often means reproduction of the entire single-celled organism. The cell grows in size, replicates its DNA, and then essentially divides into two new cells—a process called fission.



156 CHAPTER NINE



9.1 Important Consequences of Cell Division

Cell division is the basis for growth, reproduction, and regeneration.

Cell division contributes to the growth of this root tissue.



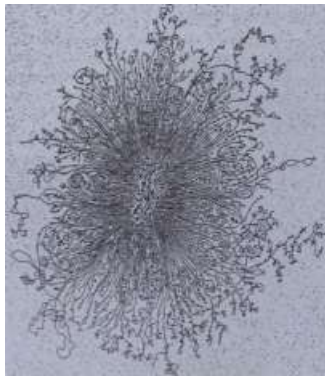
Yeast cells divide by budding. This one has nearly divided...

...and this one is beginning to bud

Cell division contributes to the regeneration of a lizard's tail.

reproductive signals. The reproductive rates of many prokaryotes respond to conditions in the environment. The bacterium *Escherichia coli*, a species that is commonly used in genetic studies, is a "cell division machine" that essentially divides continuously. Typically, cell division takes 40 minutes at 37°C. But if there are abundant sources of carbohydrates and salts available, the division cycle speeds up so that cells may divide in 20 minutes. Another bacterium, *Bacillus subtilis*, stops dividing under adverse nutritional conditions, then resumes dividing when things improve. These observations suggest that the initiation of cell division

in prokaryotes is under the control of metabolic intermediates, such as carbohydrates, in the environment.



9.2 The Prokaryotic Chromosome Is a Circle

These long, looping fibers of DNA from a cell of the bacterium *Escherichia coli* are all part of one continuous circular chromosome.

replication of dna. A chromosome, as we saw in Chapter 4, is a DNA molecule containing genetic information. When a cell divides, its chromosomes must be copied, or replicated, and each of the two resulting copies must find its way into one of the two new cells.

Most prokaryotes have only one chromosome, a single long DNA molecule with proteins bound to it. In the bacterium *E. coli*, the DNA is a circular molecule about 1.6 million nm (1.6 mm) in circumference. The bacterium itself is only about 1 μ m (1,000 nm) in diameter and about 4 μ m long. Thus the long thread of DNA, which could form a circle over 100 times larger if fully expanded, is packed into a very small space. So it is not surprising that the molecule usually appears in electron micrographs as a hopeless tangle of fibers (Figure 9.2). The DNA molecule accomplishes some packing by folding in on itself, and positively charged (basic) proteins bound to negatively charged (acidic) DNA contribute to this packing. Circular chromosomes appear to be characteristic of all prokaryotes, as well as some viruses, and are also found in the chloroplasts and mitochondria of eukaryotic cells.

Functionally, the prokaryotic DNA molecule has two regions that are important for cell reproduction:

- **Ori** is the origin of replication, where replication of the circle starts.
- **Ter** is the terminus of replication, where it ends.

The process of chromosome replication occurs as the DNA is threaded through a "replication complex" of proteins at the center of the cell.

distribution of dna. DNA replication actively drives the parceling out of the two new DNA molecules to the new cells. The first region to be replicated ison. The two ori

regions are attached to the plasma membrane, and they separate as the new chromosome forms and new plasma membrane forms between them (Figure 9.3). By the end of replication, there are two chromosomes, one at either end of the bacterial cell.

cytokinesis. Cell partition, or cytokinesis, begins 20 minutes after chromosome duplication is finished. The first event of cytokinesis is a pinching in of the plasma membrane to form a ring similar to a purse string. Fibers composed of a protein similar to eukaryotic tubulin (which makes up microtubules) are major components of this ring. As the membrane pinches in, new cell wall materials are synthesized, which finally separate the two cells.

Eukaryotic cells divide by mitosis or meiosis

Cell reproduction in eukaryotes also involves reproductive signals, DNA replication, segregation, and cytokinesis. But, as you might expect, events in eukaryotes are somewhat more complex.

First, unlike prokaryotes, eukaryotic cells do not constantly divide whenever environmental conditions are adequate. In fact, eukaryotic cells that have differentiated (become specialized) seldom divide.* So the signals for cell division are related not to the physiology of the single cell, but to the needs of the entire organism. Second, instead of a single chromosome, eukaryotes usually have many (humans have 46), so the processes of replication and segregation, while basically the same as in prokaryotes, are more intricate. Third, eukaryotic cells have a distinct nucleus, which has to be replicated and then divided into two new nuclei. Finally, cytokinesis is different in plant cells, which have a cell wall than in animal cells (which do not).

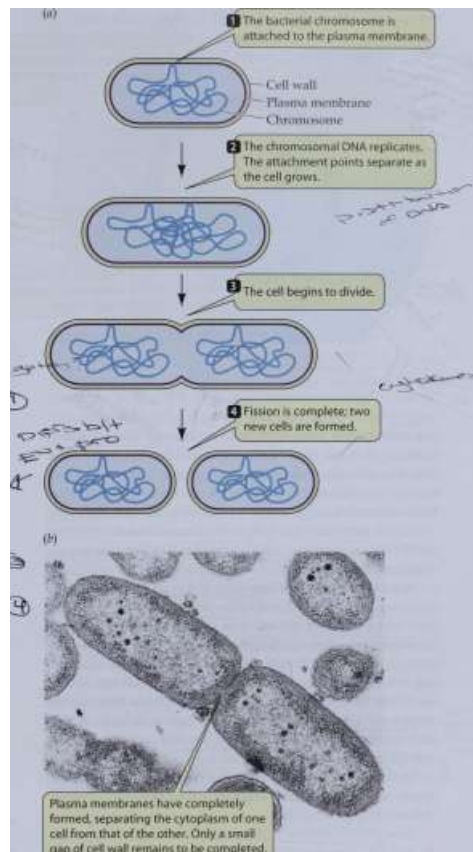
&>

mitosis is a nuclear division mechanism that operates in most types of cells. Mitosis sorts the genetic material into two new nuclei and ensures that both contain exactly the same genetic information. A second mechanism of nuclear division, meiosis, occurs in the gametes—those cells that will contribute to the reproduction of a new organism. Meiosis generalizes the shuffling of the genetic material in new gene combinations. It plays a key role in sexual life cycles.

The duplication of a eukaryotic cell typically consists of three steps:

- The replication of the genetic material within the nucleus
- The packaging and separation of the genetic material into two new nuclei
- The division of the cytoplasm

What determines whether a cell will divide? How does mitosis lead to identical cells, and meiosis to diversity? Why do we need both identical copies and diverse cells? Why do most eukaryotic organisms reproduce sexually? In the pages that follow, we will describe the details of mitosis, meiosis, and interphase, as well as their consequences for heredity, development, and evolution.



Plasma membranes have completely formed, separating the cytoplasm of one cell from that of the other. Only a small gap of cell wall remains to be completed.

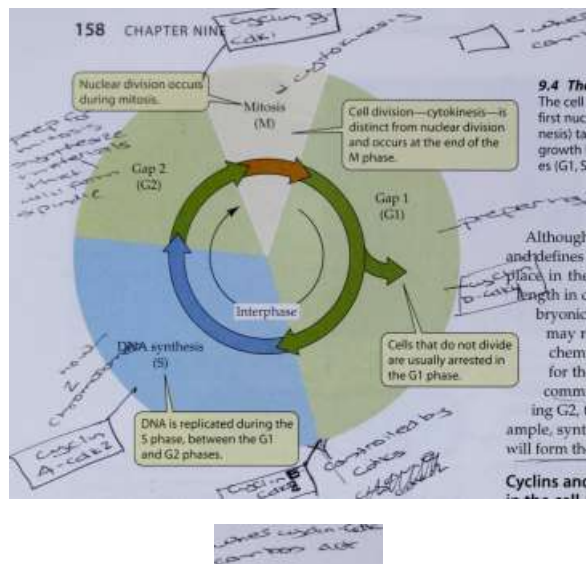
9.3 Prokaryotic Cell Division

(a) The steps of cell division in prokaryotes. (b) These two cells of the bacterium *Pseudomonas aeruginosa* have almost completed fission. Each cell contains a complete chromosome, visible as the nucleoid in the center of the cell.

Interphase and the Control of Cell Division

Between divisions of the cytoplasm—that is, for most of its life—a eukaryotic cell is in a condition called interphase. A cell lives and functions until it divides or dies—or, if it is a

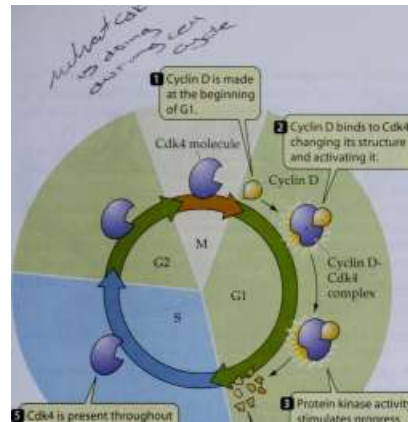
CHAPTER



G1-S transition in many other organisms, including humans.

But Cdk's are not active by themselves. They must be bound to a second type of protein, called cyclin. This binding—an example of allosteric interaction—causes the Cdk to alter its shape and exposes its active site. It is the cyclin-Cdk complex that acts as a protein kinase and triggers the transition from G1 to S phase. Then the cyclin breaks down and the Cdk becomes inactive (Figure 9.5).

Phosphorylation changes the three-dimensional structure of the targeted protein, sometimes simultaneously changing that protein's function. This important biochemical process is discussed further in Chapters 12 and 15.



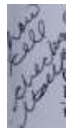
What molecules do cyclin-Cdk complexes target for phosphorylation? Some important targets are known. For example, the cyclin B-Cdk1 complex catalyzes the phosphorylation of target proteins that then bind to DNA and initiate chromosome condensation. Phosphorylation of other target proteins results in the disaggregation of the nuclear envelope early in mitosis.

Because cancer results from inappropriate cell division, it is not surprising that the cyclin-Cdk controls are disrupted in cancer cells. For example, some fast-growing breast cancers have too much cyclin D, which overstimulates Cdk4 and cell division. As we will describe in Chapter 18, a major protein in normal cells that prevents them from dividing is p53, which leads to inhibition of Cdk's. More than half of all human cancers contain defective p53, resulting in uncontrolled cell division.

Cdk4 is present throughout the cell cycle but is active only during G1.

Protein kinase activity stimulates progress through G1.

Cyclin D is broken down at the end of G1, rendering Cdk4 inactive.



9.5 Cyclin-Dependent Kinase and Cyclin Trigger Decisions in the Cell Cycle

A human cell makes the decision to enter the cell cycle during G1, when cyclin D binds to a cyclin-dependent kinase (Cdk4). There are four such cyclin-Cdk controls during the typical cell cycle in humans.

Several different cyclin-Cdk combinations act at various stages of the mammalian cell cycle:

► Cyclin D-Cdk4 acts during the mid G1 phase. This is the restriction point, a key decision point beyond which the cell cycle is normally inevitable (see Figure 9.5). —

► Cyclin E-Cdk2 acts at the G1-S boundary, initiating DNA replication.

► Cyclin A-Cdk2 acts during S, and also stimulates DNA replication.

► Cyclin B-Cdk1 acts at the G2-M boundary, initiating the transition to chromosome condensation and mitosis.

The cyclin-Cdk complexes act as checkpoints, points at which cell cycle progress can be monitored to determine if the next step can be taken. For example, if DNA is damaged by radiation during G1, a protein called p21 is made. (The p stands for "protein," and the 21 stands for its molecular weight—about 21,000 daltons.)

The p21 protein then binds to the two G1 Cdk's, preventing their activation by cyclins.

So the cell cycle stops while repairs are made to DNA. The p21 protein itself is targeted for degradation, so that it breaks down after the DNA is repaired, allowing cyclins to bind to the Cdk's and the cell cycle to proceed.

Failure of these checkpoints can lead to cancer.

Growth factors can stimulate cells to divide.

Cyclin-Cdk complexes provide an internal control for progress through the cell cycle. But there are situations in the body in which cells that are slowly cycling, or not cycling at all, must be stimulated to divide through external controls, called growth factors. When you cut yourself and bleed, specialized cell fragments called platelets gather at the wound and help initiate blood clotting. The platelets also produce and release a protein, called platelet-derived growth factor, that diffuses to the adjacent cells in the skin and stimulates them to divide and heal the wound.

Other growth factors include interleukins, which are made by one type of white blood cell and promote cell division in other cells that are essential for the body's immune system defenses. Erythropoietin, made by the kidney, stimulates the division of bone marrow cells and the production of red blood cells. In addition, many hormones promote division in specific cell types.

We will describe the physiological roles of these external mitotic inducers in later chapters, but all growth factors act in a similar way. They bind to their target cells via specialized, specific receptor proteins on the target cell surface. This specific binding triggers events within the cell that initiate a cell division cycle! Cancer cells often cycle inappropriately because they make their own growth factors, or because they no longer require growth factors to start cycling.

Eukaryotic Chromosomes

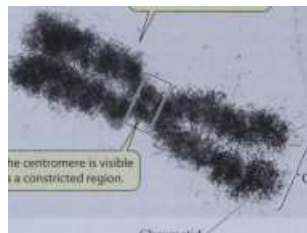
Most human cells other than eggs and sperm contain two full sets of genetic information, one from the mother and the other from the father. As in prokaryotes, this genetic information consists of molecules of DNA packaged as chromosomes. However, unlike prokaryotes, eukaryotes have more than one chromosome, and during interphase these chromosomes reside within a membrane-enclosed organelle, the nucleus.

The basic unit of the eukaryotic chromosome is a gigantic, linear, double-stranded DNA complexed

160 CHAPTER NINE

Chromatin fibers consist of DNA and proteins.

I



The centromere is visible as a constricted region.

Chromatid

Chromosome

Chromatid

9.6 Chromosomes, Chromatids, and Chromatin

A human chromosome, shown as the cell prepares to divide.

with many proteins. During most of the eukaryotic cell cycle, each chromosome contains only one such double-stranded DNA molecule. However, after the DNA molecule replicates during the S phase, the chromosome consists of two joined chromatids, each made up of one double-stranded DNA molecule complexed with proteins (Figure 9.6). The two chromatids are joined together at a specific small region called the centromere.

Chromatin consists of DNA and proteins

The complex of DNA and proteins that makes up a eukaryotic chromosome is referred to as chromatin. The DNA carries the genetic information; the proteins organize the chromosome physically and regulate the activities of the DNA. By mass, the amount of chromosomal protein is equivalent to that of DNA.

Chromatin changes form dramatically during mitosis and meiosis. During interphase, the chromatin is strung out so thinly that the chromosome cannot be seen by the light microscope. But during most of mitosis and meiosis, the chromatin is highly coiled and compacted, so that the chromosome appears as a dense, bulky object (see Figure 9.6).

This alternation of forms relates to the function of chromatin during different phases of the cell cycle. Before each mitosis, the genetic material is replicated. Mitosis separates this replicated genetic material into two new nuclei. This separation is easier to accomplish if the DNA is neatly arranged in compact units rather than being tangled up like a plate of spaghetti. During interphase, however, the DNA must direct the activities of the cell. Such functions require that portions of the DNA be unwound and exposed so that it can interact with enzymes.

Chromatin proteins organize the DNA in chromosomes

The DNA of a typical human cell has a total length of 2 meters. Yet the nucleus is only 5 μm (0.000005 meters) in diameter. So, although the DNA in an interphase nucleus is "unwound," it is still impressively packed! This packing is achieved largely by proteins associated closely with the chromosomal DNA (Figure 9.7).

During interphase, chromosomes contain large quantities of proteins called histones (from the Greek word meaning "web"). There are five classes of histones. All of them have a positive charge at cellular pH levels because of their high content of the basic amino acids lysine and arginine. These positive charges electrostatically attract the negative phosphate groups on DNA. These interactions, as well as interactions among the histones themselves, form beadlike units called nucleosomes. Each nucleosome contains:

- Eight histone molecules, two each of four of the histone classes, united to form a core or spool.
- 146 base pairs of DNA, 1.65 turns of it wound around the histone core.
- Histone H1 (the remaining histone class) on the outside of the DNA, which may clamp it to the histone core.

Interphase chromatin is made up of a single DNA molecule winding around vast numbers of nucleosomes like beads on a string. Between the nucleosomes stretches a variable amount of non-nucleosomal "linker" DNA. Since this DNA is exposed to the nuclear environment, it is accessible to proteins involved in its duplication and the regulation of its expression, as we will see in Chapter 14. There are also proteins that bind to nucleosomal DNA.

The many nucleosomes of a mitotic chromosome may pack together and coil. During both mitosis, and meiosis, the chromatin becomes ever more coiled and condensed, with further folding of the chromatin continuing up to the time at which chromosomes begin to move apart.

Mitosis: Distributing Exact Copies of Genetic Information

In mitosis, a single nucleus gives rise to two nuclei that are genetically identical and to the parent nucleus. This process ensures the accurate distribution of the eukaryotic cell's multiple chromosomes to the daughter nuclei.

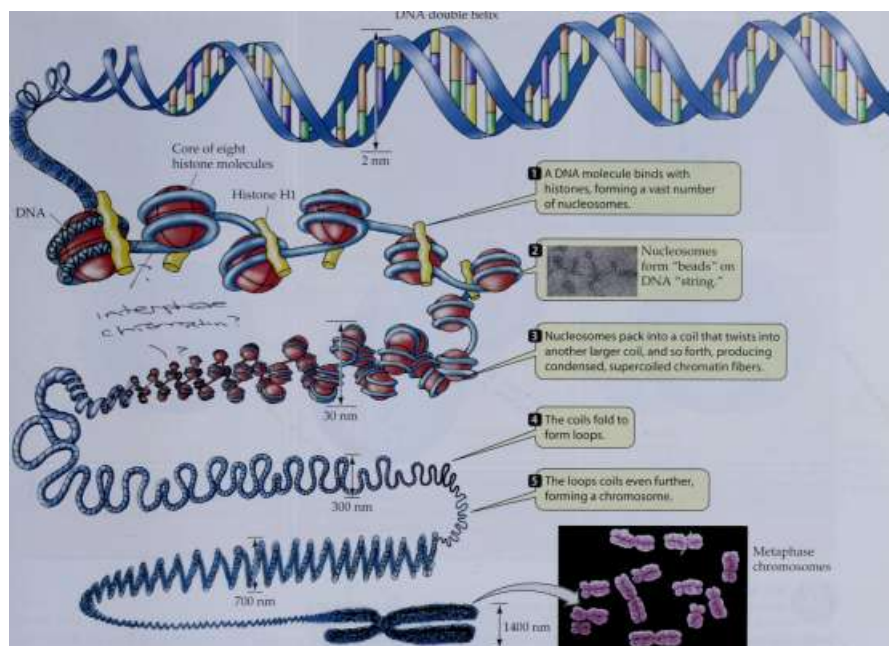
Process in which each

nucleus. In reality, mitosis is a continuous event that flows smoothly into the next. For discussion, however, it is convenient to look at mitosis—the M phase of the cell cycle—as a series of separate events, or subphases: prophase, prometaphase, metaphase, anaphase, and telophase, as shown on pages 162–163.

The centrosomes determine the plane of cell division

Once the commitment to enter mitosis has been made, the cell enters S phase, and DNA is replicated. At the same time, a pair of centrosomes ("central bodies") forms from a single centrosome that lies near the nucleus. This duplication is under the control of cyclin E-Cdk2 whose concentration peaks at the G1-to-S transition. This is the key event in orienting the direction of mitosis.

DNA double helix



Metaphase chromosomes

9.7 DNA Packs into a Mitotic Chromosome

The nucleosome, formed by DNA and histones, is the essential building block in this highly packed structure.

At the G₂-to-M transition, the two centrosomes separate from each other, moving to opposite ends of the nuclear envelope. The orientation of the centrosomes determines the plane at which the cell will divide, and therefore the spatial relationship of the two new cells to the parent cell. This relationship may be of little consequence to single free-living cells such as yeasts, but it is important for cells that make up part of a body tissue.

In many organisms, each centrosome contains a pair of centrioles. Each pair consists of one "parent" centriole and one "daughter" centriole at right angles to the parent centriole (see Figure 4.27). Their role is not clear, although they do appear to be necessary for centrosome function. Centrioles, if present, replicate during interphase: The two paired centrioles first separate, and then each acts as a "parent" for the formation of a new "daughter" centriole at right angles to it.

The centrosomes are the regions of the cell that initiate the formation of microtubules, which will orchestrate chromosomal movement, these regions are not enclosed by membranes and are not visible as discrete objects, but their positions are evident from the arrangement of nearby microtubules. Plant cells lack centrosomes, but distinct microtubule organizing centers at either end of the cell serve the same role.

The spindle forms during prophase

During interphase, only the nuclear envelope, the nucleoli, and a barely discernible tangle of chromatin are visible under the light microscope. The appearance of the nucleus changes as the replicating DNA condenses into visible chromosomes (Figure 9.8).

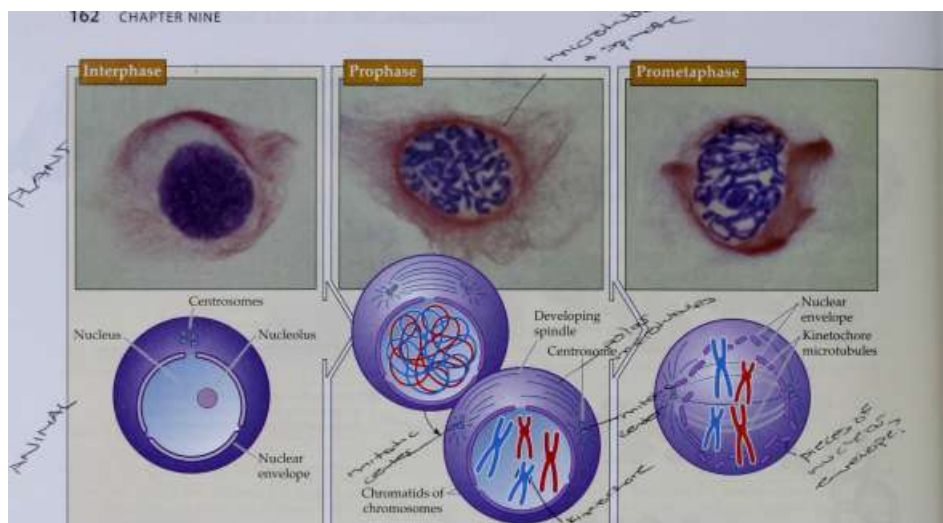
Each of the two centrosomes serves as a mitotic center that organizes microtubules. The two mitotic centers can be thought of as two poles toward which the chromosomes will move. Polar microtubules that form between the mi-

totic centers make up the developing spindle. The spindle



162 CHAPTER NINE

3^^



During the S phase of interphase, the nucleus replicates its DNA and centrosomes.

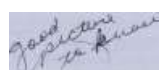
The chromatin coils and supercoils, becoming more and more compact and eventually condensing into visible chromosomes. The chromosomes consist of identical, paired chromatids.

The nuclear envelope breaks down. Kinetochore microtubules appear and connect the kinetochores with the centrosomes.



9.8 Mitosis

Mitosis results in two new nuclei that are genetically identical to one another and to the nucleus from which they formed. The photomicrographs here are of plant nuclei, which lack centrioles. The diagrams are of corresponding phases in animal cells and introduce the structures not found in plants. In the micrographs, the red dye stains microtubules (and thus the spindle); the blue dye stains the chromosomes. In the diagrams, the chromosomes are stylized to emphasize the fates of the individual chromatids.



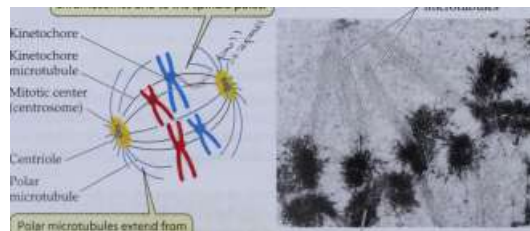
Kinetochore microtubules attach to the kinetochores in the centromeres of the chromosomes and to the spindle poles.

Kinetochore microtubules

Kinetochore

Kinetochore microtubule

Mitotic center (centrosome)



Centriole



Polar microtubules extend from («) each pole of the spindle.

(b) Kinetochore

9.9 The Mitotic Spindle Consists of Microtubules

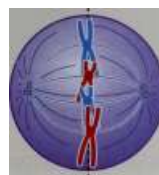
{a) Diagram of the spindle apparatus in a cell at metaphase.(b) An electron micrograph of the stage shown in (a).

CHROMOSOMES, THE CELL CYCLE, AND CELL DIVISION 163

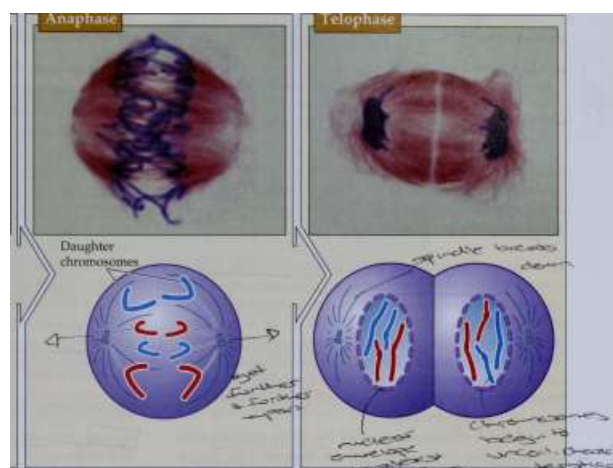
Metaphase



Equatorial (metaphase) plate



The centromere regions connecting paired chromatids become aligned in a plane at the cell's equator.



The centromere pairs separate, and the new chromosomes (each containing one member of one of the sets of paired

chromatids) begin to move toward the poles.

The separating chromosomes reach the poles. Telophase passes into the next interphase as the nuclear envelopes and nucleoli re-form and the chromatin becomes diffuse.

^3—

VO<^e-



serves as a "railroad track" along which the chromosomes will move, as well as a framework keeping the two poles apart.

The spindle is actually two half spindles: Each polar microtubule runs from one mitotic center to the middle of the spindle, where it overlaps with polar microtubules of the other half spindle (Figure 9.9). The polar microtubules are initially unstable, constantly forming and falling apart, until they contact polar microtubules from the other half spindle and become more stable.

A prophase chromosome consists of two chromatids

The chromatin also changes during prophase. The extremely long, thin fibers take on a more orderly form as a result of coiling and compacting (see Figures 9.7 and 9.8). Under the light microscope, each prophase chromosome can be seen to consist of two chromatids held tightly together over much of their length. The two chromatids of a single mitotic chromosome are identical in structure, chemistry, and the hereditary information they carry because of the way in which DNA replicates during the S phase.

Within the region of tight binding of the chromatids lies the centromere, which is where chromatids become associ-

<IX~

ated with the microtubules of the spindle. Very late in prophase, specialized three-layered structures called kinetochores develop in the centromere region, one on each chromatid (see Figure 9.9). The kinetochores are the sites at which microtubules will attach to the chromatids.

Chromosome movements are highly organized

The next three phases of mitosis—prometaphase, metaphase, and anaphase—are the phases during which chromosomes actually move. During these phases, the centromeres; holding the two chromatids together separately, and the former chromatids—now called daughter chromosomes—move away from each other in opposite directions.

prometaphase. At the beginning of prometaphase, the nuclear lamina disintegrates and the nuclear envelope breaks into small vesicles, allowing the developing spindle to "invade" the nuclear region. The polar microtubules begin to attach to chromatids at their kinetochores, at which point they are called kinetochore microtubules (see Figure 9.9). The kinetochore of one chromatid is attached to microtubules coming from one pole, while the kinetochore of its sister chromatid is attached to microtubules emanating from the other pole.

164 CHAPTER NINE

During prometaphase, the movement of chromosomes toward the poles is counteracted by two factors:

the United States! This slow speed may ensure that the chromosomes segregate accurately.

£-'

A repulsive force from the poles pushes the chromosomes toward the middle region, or equatorial

(metaphase) plate, between the poles. *~~

The two chromatids are held together, apparently by proteins called cohesins.

So, during prometaphase, chromosomes appear to move aimlessly back and forth between the poles and the middle of the spindle. Gradually, the kinetochores approach the equatorial plate (see Figure 9.9).

metaphase. The cell is said to be in metaphase when all the kinetochores arrive at the equatorial plate. Metaphase lasts up to an hour, and is the best time to see the sizes and shapes of chromosomes. Because a microtubule (or a bundle of them) from one of the poles is attached to one of the kinetochores in each chromosome at its kinetochore (and chromatid) is oriented toward that pole. By default, the other kinetochore faces the other pole, and becomes attached to that pole's microtubule(s).

At the end of metaphase, all of the chromatids separate simultaneously. Two things appear to happen first, the cohesins break down, and then, an enzyme called DNA topoisomerase II unravels the interconnected DNA's at the cen-

**Nuclei re-form during telophase

^^^rWhen the chromosomes stop moving at the end of ana-

phase, the cell enters telophase. Two sets of chromosomes containing identical DNA, carrying identical sets of hereditary instructions, are now at the opposite ends of the spindle, which begins to break down. The chromosomes begin to uncoil, continuing until they become the diffuse tangle of chromatin that is characteristic of interphase. The nuclear envelopes and nucleoli, which were disaggregated during prephase, reappear and re-form their respective structures. When these and other changes are complete, telophase—and cytokinesis—is at an end, and each of the daughter nuclei enters another interphase.

Mitosis is beautifully precise. Its result is two nuclei that are identical to each other and to the parent nucleus in chromosome number, and hence in genetic constitution.

Cytokinesis: The Division of the Cytoplasm



Mitosis refers only to the division of the nucleus. The division of the cell's cytoplasm, which follows mitosis, is called cytokinesis.

Anaphase. Separation of the chromatids marks the beginning of anaphase, the phase of mitosis during which the two sister chromatids of each chromosome are now called daughter chromosomes, each containing one double-stranded DNA molecule—move to opposite ends of the spindle.

Completed by cytokinesis

Animal cells usually divide by a furrowing of the plasma membrane, as if an invisible thread were tightening between the two poles (Figure 9.10f). The invisible thread is actually microfilaments of actin and myosin (see Figure 4.23a) located in a ring just beneath the plasma membrane. These two proteins interact to produce a contraction, just as they do in muscles, thus pinching the cell in two. These microfilaments assemble rapidly from actin monomers that are present in the interphase cytoskeleton. Their assembly appears to be under the control of Ca^{2+} released from storage sites in the center of the cell. Plant cells divide differently, because plants

► In the

What propels this highly organized mass migration, which takes about 10 minutes, is not clear. Two things seem to move the chromosomes along. First, at the kinetochores are proteins that act as "molecular motors." These proteins, called cytoplasmic dynein, have the ability to hydrolyze ATP and have cell walls. As the spindle breaks down after mitosis, g^{+} SJADP and phosphatase, thus releasing energy to move the membranous vesicles derived from the Golgi apparatus ap-

Chromosomes along the microtubules toward the poles. appear in the equatorial region roughly midway between the

These motor proteins account for about 75 percent of the two daughter nuclei. Moving along microtubules, these

force of motion. Second, the kinetochore microtubules and vesicles fuse to form new plasma membrane and contribute

their contents to a cell plate, which is the beginning of a new cell wall (Figure 9.10g)

•c*

shorten from the poles, drawing the chromosomes toward them This accounts for about 25 percent of the motion.

During anaphase the poles of the spindle are pushed farther apart, doubling the distance between them. The distance between poles increases because polar microtubules from opposite ends of the spindle contain motor proteins that cause them to slide past each other, pushing the poles apart in much the same way that microtubules slide in cilia and flagella (see Chapter 4). This polar separation contributes to the separation of one set of daughter chromosomes from the other.

The movements of chromosomes are slow, even in cellular terms. At about 1 Km per minute, it takes about 10-60 minutes for them to complete their journey to the poles. This is like a human taking 7 million years to travel across

Following cytokinesis, both daughter cells contain all the components of a complete cell. A precise distribution of chromosomes is ensured by mitosis. Organelles such as ribosomes, mitochondria, and chloroplasts need not be distributed equally between daughter cells as long as some of each are present in both cells; accordingly, there is no mechanism with a precision comparable to that of mitosis to provide for their equal allocation to daughter cells.

Reproduction: Sexual and Asexual

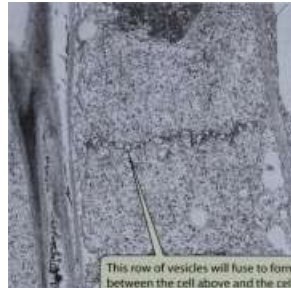
The mitotic cell cycle repeats itself. By this process, a single cell can give rise to a vast number of others. Meiosis, on the

(«)

The division furrow has completely separated the cytoplasm of one daughter cell from another, although their surfaces remain in contact.



(b)



Microtubules

between the cell above and the cell below

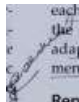
cell plate? low.

other hand, results in only four progeny, which usually do not undergo further duplication. These two methods of nuclear and cell division have different reproductive roles.

Reproduction by mitosis results in genetic constancy

A single cell that undergoes mitosis may be an entire organism reproducing itself with each cell cycle, or a cell that divides further to produce a multicellular organism. A multicellular organism, in turn, may be able to reproduce itself by releasing cells derived from mitosis and cytokinesis as a spore, or by having a multicellular piece break away and grow on its own (Figure 9.11).

A dividing unicellular organism and a multicellular organism reproducing by releasing cells both provide examples of asexual reproduction, sometimes called vegetative reproduction. This mode of reproduction is based on mitotic division of the nucleus and produces a clone of offspring that are genetically identical to the parent. If



9.11 Asexual Reproduction

These spool-shaped cells are asexual spores formed by a fungus. Each spore contains a nucleus produced by a mitotic division. A spore is the same genetically as the parent that fragmented to produce it.

9.10 Cytokinesis Differs in Animal and Plant Cells

Plant cells form cell walls and thus must divide differently from animal cells, (a) A sea urchin egg that has just completed cytokinesis at the end of the first cell division of its development into an embryo. (b) A dividing plant cell in late telophase.

there is any variation among the offspring, it is likely to be due to mutations, or changes, in the genetic material. Asexual reproduction is a rapid and effective means of making new individuals, and it is common in nature.

Sexual reproduction, which involves meiosis, is very different. In sexual reproduction two parents, each contributing one cell, produce offspring that differ genetically from

parent as well as from each other. This variety among the offspring means that some of them may be better adapted than others to reproduce in a particular environment.

Sexual reproduction, which combines genetic information from two different cells, fosters genetic diversity. The hallmarks of all sexual life cycles are:

Haploid cells contain only one homolog from each pair of chromosomes. The number of chromosomes in such a single set is denoted by n . When haploid gametes fuse in fertilization, the resulting zygote has two homologs of each type. It is thus said to be diploid, denoted $2n$.

As you can see in Figure 9.12, sexual life cycles exhibit different patterns of development after zygote formation. In haplontic organisms, such as protists and many fungi, the mature organism is haploid. The zygote undergoes a reduction division—meiosis—to produce haploid cells, or spores. These spores then form the new organism by mitosis of haploid cells, which may be single-celled or multicellular. Gametes are then produced by this organism by mitosis. So in haplontic organisms, the zygote is the only diploid cell in the life cycle.

At the other extreme are eukaryotic organisms, which include animals and some plants. Here, the gametes are the "only" haploid cells, and the organism itself is diploid. Gametes are formed by meiosis, and the formation of the organism involves mitosis of diploid cells.

Gametes are formed by meiosis, and the formation of the organism involves mitosis of diploid cells.

9.72 Fertilization and Meiosis Alternate in Sexual Reproduction

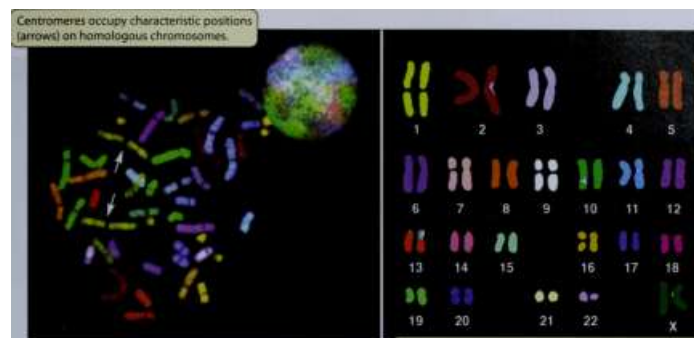
In sexual reproduction, haploid (n ; yellow) cells or organisms alternate with diploid ($2n$; blue) cells or organisms.

In the middle are organisms that have an alternation of

haploid and diploid generations. Most plants fall into this category. Here, the zygote divides by mitosis of diploid cells into a diploid organism. Meiosis does not give rise to gametes, but instead to haploid spores that divide by mitosis to form an alternate, haploid life stage. It is this haploid organism that forms gametes by mitosis, and after fertilization the cycle begins anew. We will look at all of these life cycles in greater detail in subsequent chapters.

The essence of sexual reproduction is the random selection of half of a parent's diploid chromosome set to make a haploid gamete by the fusion of two such haploid gametes to produce a diploid cell that contains genetic information from both gametes. Both of these steps contribute to a shuffling of genetic information in the population, so no two individuals have exactly the same genetic constitution. The diversity provided by sexual reproduction opens up enormous opportunities for evolution.

Centromeres occupy characteristic positions (arrows) on homologous chromosomes.



9.73 Human Cells Have 46 Chromosomes

Chromosomes from a human cell are shown in metaphase of mitosis. In this "chromosome painting" technique, each homologous pair shares a distinctive color. The multicolored globe is an interphase nucleus. The karyotype on the right is produced by computerized analysis of the image on the left.

The number, shapes, and sizes of the metaphase chromosomes constitute the karyotype.

When nuclei are in metaphase of mitosis, it is often possible to count and characterize the individual chromosomes. This is a relatively simple process in some organisms, thanks to techniques that can capture cells in metaphase and spread out the chromosomes. A photograph of the entire set of chromosomes can then be made, and the images of the individual chromosomes can be placed in an orderly arrangement. Such a rearranged photograph reveals the

The karyotype shows 23 pairs of chromosomes, including the sex chromosomes. This female's sex chromosomes are X and X; a male would have X and Y chromosomes.

number, shapes, and sizes of chromosomes in a cell, which together constitute its karyotype (Figure 9.13).

Individual chromosomes can be recognized by their lengths, the positions of their centromeres, and characteristic banding when they are stained and observed at high magnification. When the cell is diploid, the karyotype consists of homologous pairs of chromosomes—23 pairs for a total of 46 chromosomes in humans, and greater or smaller numbers of pairs in other diploid species. There is no simple relationship between the size of an organism and its chromosome number (Table 9.1).

Meiosis: A Pair of Nuclear Divisions

Meiosis consists of two nuclear divisions that reduce the number of chromosomes to the haploid number in preparation for sexual reproduction. Although the nucleus divides twice during meiosis, the DNA is replicated only once. To understand the process of meiosis and its specific details, it is useful to keep in mind the overall functions of meiosis:

- To reduce the chromosome number from diploid to haploid.
- To ensure that each of the haploid products has a complete set of chromosomes.
- To promote genetic diversity among the products.

Two unique features characterize the first meiotic division, meiosis I. The first is that homologous chromosomes pair along their entire lengths. This process, called synapsis, lasts from prophase to the end of metaphase. The second is that after this metaphase, the homologous chromosomes separate. The individual chromosomes, each consisting of two joined sister chromatids, remain intact until the end of the metaphase of meiosis II, the second meiotic division. In the discussion that follows, you can refer to Figure 9.14 to help you visualize each step.

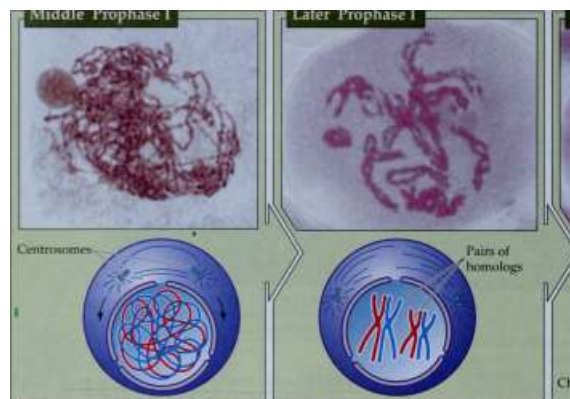
168 CHAPTER NINE

^

MEIOSIS I

Middle Prophase I

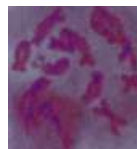
Later Prophase I



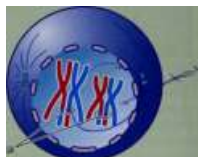
The chromatin begins to condense following interphase.

Synapsis aligns homologs, and chromosomes condense. Homologs are shown in different colors indicating those coming from each parent. In reality, their differences are very small, usually comprising different alleles of some genes.

Late Prophase I-Prometaphase

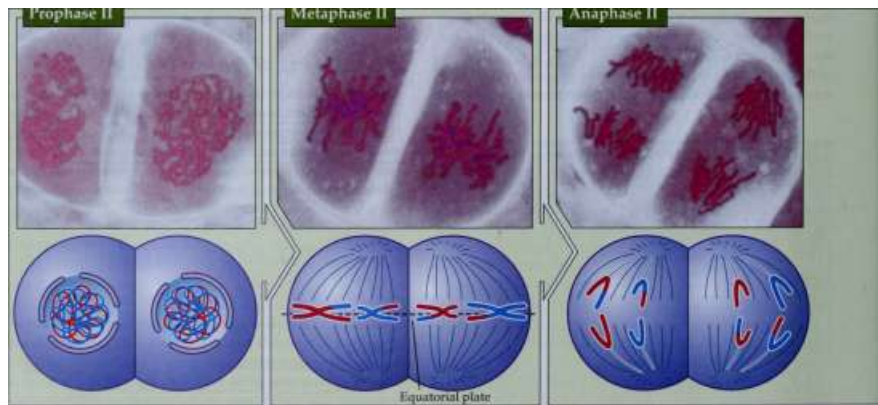


Chiasmata



The chromosomes continue to coil and shorten. Crossing-over at chiasmata results in an exchange of genetic material. In prometaphase the nuclear envelope breaks down.

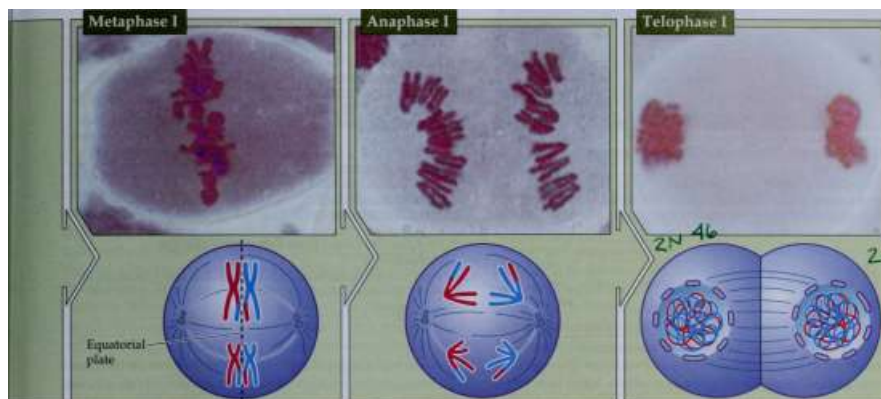
MEIOSIS II



The chromosomes condense again, following a brief interphase (interkinesis) in which DNA does not replicate.

Kinetochores of the paired chromatids line up across the equatorial plates of each cell.

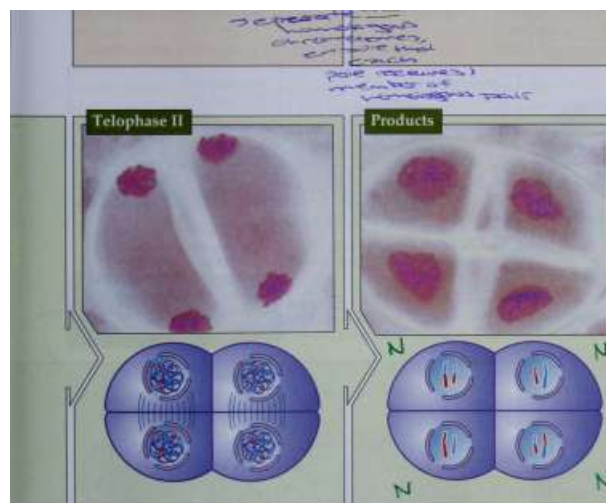
The chromatids finally separate, becoming chromosomes in their own right, and are pulled to opposite poles. Because of crossing over in prophase I, each new cell will have a different genetic makeup.



1L

The chromosomes line up on the equatorial (metaphase) plate.

The homologous chromosomes (each with two chromatids) move to opposite poles of the cell.



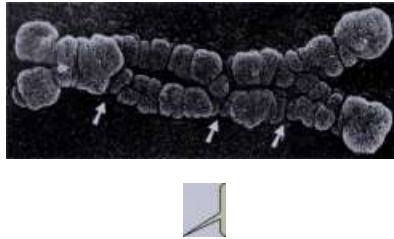
The chromosomes gather into nuclei, and the original cell divides.



The chromosomes gather into nuclei, and the cells divide.

Each of the four cells has a nucleus with a haploid number of chromosomes.

9.14 Meiosis In meiosis, two sets of chromosomes are divided among four nuclei, each of which then has half as many chromosomes as the original cell. These four haploid cells are the result of two successive nuclear divisions. The photomicrographs shown here are of meiosis in the male reproductive organ of a lily. As in Figure 9.8, the diagrams show corresponding phases in an animal.



During prophase I, homologous chromosomes, each with a pair of sister chromatids, line up to form a tetrad.

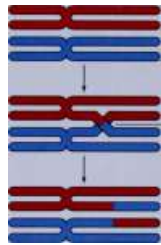
9.7 5 Chiasma at a: Evidence of Exchange between Chromatids

Chiasmata are visible near the middle of some chromatids from a desert locust in this scanning electron micrograph, and near the ends of others. Three chiasmata are indicated with arrows.

The first meiotic division reduces

the chromosome number - ^ ^

Like mitosis, meiosis I is preceded by an interphase with an S phase during which $2n$ chromosomes are replicated. As a result, each chromosome consists of two sister chromatids. Meiosis I begins with a long prophase I (the first three frames of Figure 9.14), during which the chromosomes change markedly. A key change is that homologous chromosomes join together, or synapse. By the time they can be clearly seen under light microscope, the two homologs are



li\ Homologous ■1/ chromosomes U

A chiasma forms between adjacent chromatids of different homologs.

y --

Chiasma

I Breakage and rejoining at the chiasma results in recombinant chromatids.

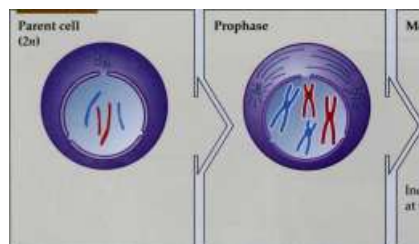
~| Recombinant J chromatids

9.7 6 Crossing Over Forms Genetically Diverse Chromo~somes

The exchange of genetic material by crossing over may result in new combinations of genetic information on the recombinant chromosomes.

already tightly joined. This joining begins at the centromeres and is mediated by a recognition of homologous DNA sequences on homologous chromosomes. In addition, a special group of proteins may form a scaffold called the synaptonemal complex that runs lengthwise along the homologous chromosomes and appears to join them together. The

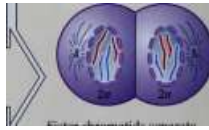
MITOSIS



Metaphase



Two daughter cells (each $2n$)



Individual chromosomes align at the equatorial (metaphase) plate.

Sister chromatids separate during anaphase, becoming daughter chromosomes.

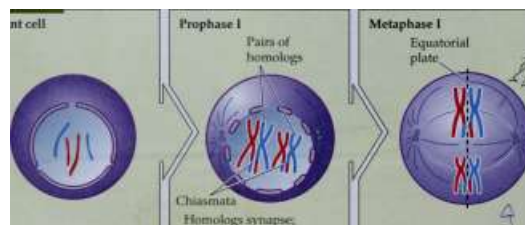
Mitosis is a mechanism for constancy: The parent nucleus produces two daughter nuclei, identical to the parent and to each other.

MEIOSIS

Parent cell ($2m$)

Metaphase I

Equatorial plate s



Interkinesis

Chiasmata

Homologs synapse; chiasmata form (at least one per pair of homologs).



Centromeres do not separate; sister chromatids remain together during Homolog pairs align at the ^, - ^kaphase; homologs separate; QJkLA-does equatorial plate. : ^ "not replicatg, before subsequent prophase.



four chromatids of each pair of homologous chromosomes form a tetrad or bivalent. To summarize: A tetrad is four chromatids, two each from two homologous chromosomes. For example, there are 46 chromosomes in a human diploid cell, so there are 23 homologous pairs of chromosomes, each with two chromatids, for a total of 92 chromatids during prophase I. In other words, there are 23 tetrads, each containing two homologous chromosomes and four chromatids.

Throughout prophase I and metaphase I, the chromatin continues to coil and compact progressively, so the chromosomes appear ever thicker. At a certain point, the homologous chromosomes seem to repel each other, especially near the centromeres, but they are held together by physical attachments. Regions having these attachments take on an X-shaped appearance and are called chiasmata (from the Greek word "chiasma," meaning "cross"; Figure 9.15). A chiasma reflects an exchange of material between chromatids on homologous chromosomes—what geneticists call crossing over (Figure 9.16). The chromosomes begin exchanging material shortly after synapsis begins, but the chiasmata do not become visible until later, when the homologs are repelling each other.

Crossing over increases the genetic variation among the daughter cells. At the end of a mitotic division has. prokaryotes; We will have a great deal in synapsis in some species, but not in others, there is a telophase I,

Prophase I is followed by metaphase I (not pictured in Figure 9.14), during which the nuclear envelope and the nucleoli disappear. A spindle forms, and microtubules become attached to the kinetochores of the chromosomes. In meiosis I, there is only one kinetochore per chromosome, not one per chromatid as in mitosis. Thus the entire chromosome, consisting of two chromatids, will migrate to one pole of the meiotic cell.

By metaphase I, all the chromosomal kinetochores have become connected to microtubules, and all the chromosomes have moved to the equatorial plate. Until this point, they have been held together by chiasmata.

The homologous chromosomes separate in anaphase I, when individual chromosomes, each still consisting of two chromatids, are pulled to the poles, with one homolog of a

pair going to one pole and the other homolog to the opposite pole. (Note that this process differs from the separation of chromatids during mitotic anaphase.) Each of the two daughter nuclei from this division is haploid; that is, it contains only one set of chromosomes, not the two sets that were present in the original diploid nucleus. However, because they consist of two chromatids rather than just one, each of these chromosomes has twice the mass that a chro-

some has. We will discuss crossing over and its genetic consequences in the coming chapters.

There seems to be plenty of time for the complicated events of prophase I to occur. Whereas mitotic prophase is usually measured in minutes, and all of mitosis seldom takes more than an hour or two, meiosis can take much longer. In human males, the cells in the testis that undergo "meiosis" take about a week for prophase I and about a month for the entire meiotic cycle. In the cells that will become eggs, prophase I begins long before a woman's birth, during early fetal development, and ends as much as decades later during the monthly ovarian cycle.

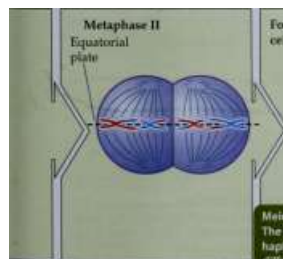
9.7 Mitosis and Meiosis: A Comparison

Meiosis differs from mitosis by synapsis and by the failure of the centromeres to separate at the end of metaphase I. - k

/

Metaphase II

Equatorial plate



Four daughter cells (each n)

\1

;



Chromatids separate.

Meiosis is a mechanism for diversity: The parent nucleus produces four haploid daughter nuclei, each different from the parent and from its sisters.

with the reappearance of nuclear envelopes and so forth. When there is a telophase I, it is followed by an interphase, called interkinesis, similar to the mitotic interphase. During interkinesis the chromosome is partially uncoiled; however, there is no replication of the genetic material because each chromosome already consists of two chromatids. Furthermore, the sister chromatids in interkinesis are generally not genetically identical, because crossing over in prophase I has reshuffled genetic material between maternal and paternal chromosomes.

The second meiotic division separates the chromatids

Meiosis II is similar to mitosis in many ways. In each nucleus produced by meiosis I, the chromosomes line up at equatorial plates in metaphase II, the chromatids—each of which has a centromere—separate, and new daughter chromosomes move to the poles in anaphase II.

The three major differences between meiosis II and mitosis are:

- DNA replicates before mitosis, but not before meiosis II. 1

► In mitosis, the sister chromatids that make up a given chromosome are identical; in meiosis II, they differ over part of their length if they participated in crossing over during prophase of meiosis I.

► /The number of chromosomes on the equatorial plate of each of the two nuclei in meiosis II is half the number in the single mitotic nucleus!

Figure 9.17 compares mitosis and meiosis. The result of meiosis is four nuclei: each nucleus is haploid and has a single set of chromosomes that differs from other such sets

172 CHAPTER NINE



in its exact genetic composition. The differences, to repeat a very important point, result from crossing over during prophase I and from the segregation of homologous chromosomes during anaphase I.

Meiosis leads to genetic diversity

What are the consequences of the synapsis and separation of homologous chromosomes during meiosis? In mitosis, each chromosome behaves independently of its homolog; its two chromatids are sent to opposite poles at anaphase. If we start a mitotic division with x chromosomes, we end up with x chromosomes in each daughter nucleus, and each chromosome consists of one chromatid. In meiosis, things are very different.

In meiosis, synapsis organizes things so that chromosomes of maternal origin pair with their paternal homologs. Then their separation during meiotic anaphase I ensures that each pole receives one member of each homologous pair. (Remember that each chromosome still consists of two chromatids.) For example, at the end of meiosis I in humans, each daughter nucleus contains 23 of the original 46 chromosomes. In this way, the chromosome number is decreased from diploid to haploid. Furthermore, meiosis I guarantees that each daughter nucleus gets one full set of chromosomes, for it must have one of each homologous pair.

which one or more chromosomes or pieces of chromosomes are either lacking or present in excess.

Aneuploidy can give rise to genetic abnormalities

One reason for nondisjunction may be a lack of chiasmata. Recall that these structures, formed during prophase I, hold the two homologous chromosomes together into metaphase I. This ensures that one homolog will face one pole and the other homolog the other pole. Without this "glue," the two homologs may line up randomly at metaphase I, just like chromosomes during mitosis, and there is a 50 percent chance that both will go to the same pole. If, for example, the chromosome 21 pair fails to separate during the formation of a human egg (and thus both mem-

| Only one pair of homologous chromosomes is emphasized. In humans, there are a total of 22 other pairs.

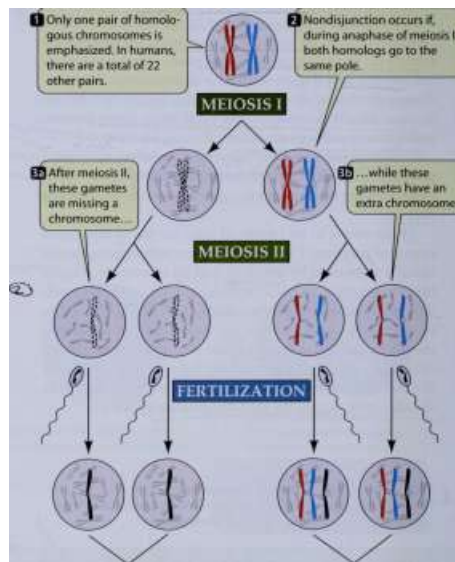
Nondisjunction occurs if, during anaphase of meiosis I, both homologs go to the same pole.

The products of meiosis I are genetically diverse for two reasons. First, synapsis during prophase I allows the maternal chromosome to interact with the paternal one; after crossing over, the recombinant chromatids contain some genetic material from each chromosome. Second, which member of a homologous pair goes to which daughter cell at anaphase I is a matter of probability. For example, if there are two pairs of chromosomes in the diploid parent nucleus, a particular daughter nucleus could get paternal chromosome 1 and maternal chromosome 2, or paternal 2 and maternal 1, or both maternals, or both paternals. It all depends on the way in which the homologous pairs line up at metaphase I.

Note that of the four possible chromosome combinations just described, two produce daughter nuclei that are the same as one of the parental types (except for any material exchanged by crossing over). The greater the number of chromosomes, the less probable that the original parental combinations will be reestablished, and the greater the potential for genetic diversity. Most species of diploid organisms do, indeed, have more than two pairs. In humans, with 23 chromosome pairs, 2²³ different combinations can be produced.

Meiotic Errors

A pair of homologous chromosomes may fail to separate during meiosis I, or sister chromatids may fail to separate during meiosis II or during mitosis. This phenomenon is called nondisjunction and it results in the production of aneuploid cells (Figure 9.18). Aneuploidy is a condition in



Chromosome from normal gamete

ff Fertilization with a gamete containing the normal number of chromosomes (23) results in an individual with one chromosome missing (monosomy).

Chromosome from normal gamete

QJ Fertilization with a gamete containing the normal number of chromosomes (23) results in an individual with an extra chromosome.

9.18 Nondisjunction Leads to Aneuploidy

Nondisjunction occurs if homologous chromosomes fail to separate during meiosis I. The result is aneuploidy: One or more chromosomes are either lacking or present in excess.

bers of the pair go to one pole during anaphase I), the resulting egg will contain either two of chromosome 21 or none at all. If an egg with two of these chromosomes is fertilized by a normal sperm, the resulting zygote will have three copies of the chromosome: It will be trisomic for chromosome 21. A child with an extra chromosome 21 demonstrates the symptoms of Down syndrome: impaired intelligence; characteristic abnormalities of the hands, tongue, and eyelids; and an increased susceptibility to cardiac abnormalities and diseases such as leukemia.

Other abnormal events can also lead to aneuploidy. In a process called translocation, a piece of a chromosome may break away and become attached to another chromosome. For example, a particular large part of one chromosome 21 may be translocated to another chromosome. Individuals who inherit this translocated piece along with two normal chromosomes 21 will have Down syndrome.

Trisomies (and the corresponding monosomies) are surprisingly common in human zygotes, but most of the embryos that develop from such zygotes do not survive to birth. Trisomies for chromosomes 13, 15, and 18 greatly reduce the probability that an embryo will survive to birth.

Actually, all infants who are born with such trisomies die before the age of 1 year.

(Trisomies and monosomies for other chromosomes are lethal to the embryo. About one-fifth of all recognized pregnancies spontaneously terminate during the first two months, largely because of such trisomies and monosomies. (The actual proportion of spontaneously terminated pregnancies is certainly higher, because the earliest ones often go unrecognized.)

Polyoids can have difficulty in cell division

Both diploid and haploid cells divide by mitosis. Multicellular diploid and multicellular haploid individuals develop from single-celled beginnings by mitotic division. Likewise, mitosis may proceed in diploid organisms even when a chromosome from one of the haploid sets is missing or when there is an extra copy of one of the chromosomes (as in Down syndrome).

Under some circumstances, triploid (3n), tetraploid (4n), and higher-order polyploid nuclei may form. Each of these

ploidy levels represents an increase in the number of complete sets of chromosomes present. If, by accident, the nucleus has one or more extra full sets of chromosomes—that is, if it is triploid, tetraploid, or of still higher ploidy—this abnormally high ploidy in itself does not prevent mitosis. Nonetheless, each chromosome behaves independently of the others.

In meiosis, by contrast, chromosomes synapse to begin division. If even one chromosome has no homolog, anaphase I cannot send representatives of that chromosome to both poles. A diploid nucleus can undergo normal meiosis; a haploid one cannot. A tetraploid nucleus has an even number of each kind of chromosome, so each chromosome can pair with its homolog. But

a triploid nucleus cannot undergo normal meiosis, because one-third of the chromosomes would lack partners.

This limitation has important consequences for the fertility of triploid, tetraploid, and other chromosomally unusual organisms that may be produced by plant breeding or by natural accidents. Modern bread wheat plants are hexaploids, the result of the accidental crossing of three different grasses, each having its own diploid set of 14 chromosomes.

Cell Death

As we mentioned at the start of this chapter, an essential role of cell division in complex eukaryotes is to replace cells that die. In humans, billions of cells die each day, mainly in



in

the blood and the epithelial lining organs such as the intestine. Cells die in one of two ways. The first necrosis occurs when cells either are damaged by poisons or are starved of essential nutrients. These cells usually swell up and burst, releasing their contents into the extracellular environment. This often results in inflammation (see Chapter 19). The scab that forms around a wound is a familiar example of necrotic tissue.

More typically, cell death in an organism is due to apoptosis (from the Greek word meaning "falling of"). Apoptosis is a prescribed event that constitutes genetically programmed cell death. These two ways for cells to die are compared in Table 9.2.

Two Different Ways for Cells to Die

NECROSIS

APOPTOSIS

Stimuli

ATP required Cellular pattern

DNA breakdown Plasma membrane Fate of dead cells Reaction in tissue

Low O_2 , toxins, ATP depletion, damage

No

Swelling, organelle disruption,

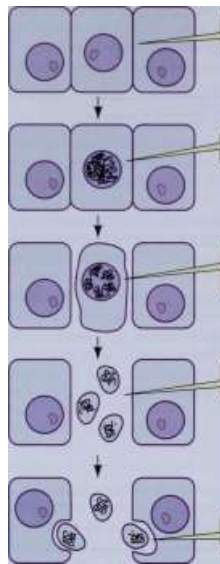
tissue death Random fragments Burst

Ingested by phagocytes Inflammation

Specific, genetically programmed physiological

signals Yes Chromatin condensation, membrane blebbing,

single-cell death Nucleosome-sized fragments Blebbed (see Figure 9.19f) Ingested by neighboring cells No inflammation



A normal cell is in contact with its neighbors.

Chromatin in an apoptotic cell begins to condense.

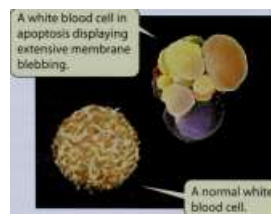
The cell detaches from its neighbors and the chromatin fragments.

The cell breaks up into fragments.

Surrounding cells ingest the fragments.

(«)

A white blood cell in apoptosis displaying extensive membrane blebbing.



(b)

9.19 Apoptosis: Programmed Cell Death

Many cells are genetically programmed to die when they are no longer needed, or when they have lived long enough to accumulate a burden of DNA damage that might harm the organism.

Why would a cell initiate apoptosis, which is essentially "cell suicide"? One reason is that the cell in question is no longer needed by the organism. For example, before birth, a human fetus has weblike hands, with connective tissue between the fingers. As development proceeds, this unneeded tissue disappears as its cells undergo apoptosis (see Figure 16.10).

A second reason for apoptosis is that the longer cells live, the more prone they are to damage that could lead to cancer. This is especially true of cells in the blood and intestine, which are exposed to high levels of toxic substances. In these cases, cells "sacrifice their lives for the good of the organism." Such cells normally die after only days or weeks.

Like the cell division cycle, the cell death cycle has signals controlling its progress. These include the lack of a mitogenic signal, such as a growth factor, and recognition of DNA damage. As we will see in Chapter 17, many of the drugs used to treat diseases of cell proliferation such as cancer work via these signals.

The events of apoptosis are very similar in most organisms (Figure 9.19). The cell becomes isolated from its neighbors, chops up its chromatin into nucleosome-sized pieces, and then fragments itself. In a remarkable example of the economy of nature, the surrounding living cells usually ingest the remains of the dead cell. The genetic signals that lead to apoptosis are also common to many organisms.

Chapter Summary

► Cell division is necessary for reproduction, growth, and repair of an organism. Review Figure 9.1

Systems of Cell Reproduction

► Cell division must be initiated by a reproductive signal. Cell division consists of three steps: replication of the genetic material (DNA), partitioning of the two DNA molecules to separate portions of the cell, and division of the cytoplasm.

► In prokaryotes, cellular DNA is a single molecule, or chromosome. Prokaryotes reproduce by cell fission. Review Figure 9.3

► In eukaryotes, nuclei divide by either mitosis or meiosis.

Interphase and the Control of Cell Division

► The mitotic cell cycle has two main phases: interphase (during which cells are not dividing) and mitosis (when cells divide).

► During most of the cell cycle the cell is in interphase, which is divided into three subphases: S, G₁, and G₂. DNA is replicated during S phase. Review Figure 9.4

► Cyclin-Cdk complexes regulate the passage of cells from G₁ into S phase and from G₂ into M phase. Review Figure 9.5

► In addition to the internal cyclin-Cdk complexes, controls external to the cell, such as growth factors and hormones, can also stimulate the cell to begin a division cycle.

Eukaryotic Chromosomes

► Chromosomes contain DNA and proteins. At mitosis, chromosomes initially appear to be double because two sister chromatids are held together at the centromere. Each sister chromatid consists of one double-stranded DNA molecule complexed with proteins and referred to as chromatin. Review Figure 9.6

► During interphase, the DNA in chromatin is wound around cores of histones to form nucleosomes. DNA folds over and over again, packing itself within the nucleus. When mitotic chromosomes form, it folds even more. Review Figure 9.7

CHROMOSOMES, THE CELL CYCLE, AND CELL DIVISION 175

Mitosis: Distributing Exact Copies of Genetic Information

► After DNA is replicated during S phase, the first sign of mitosis is the separation of centrosomes, which initiate microtubule formation for the spindle. Review Figure 9.9

► Mitosis can be divided into several phases, called prophase, prometaphase, metaphase, anaphase, and telophase. Review Figure 9.8

► During prophase, the chromosomes condense and appear as paired chromatids.

► During prometaphase, the chromosomes move toward the middle of the spindle. In metaphase, they gather at the middle of the cell with their centromeres on the equatorial plate. At the end of metaphase, the centromeres holding the chromatid pairs together separate, and during anaphase each member of the pair, now called a daughter chromosome, migrates to its pole along the microtubule track.

► During telophase, the chromosomes become less condensed. The nuclear envelopes and nucleoli re-form, thus producing two nuclei whose chromosomes are identical to each other and to those of the cell that began the cycle. Review Figure 9.8

Cytokinesis: The Division of the Cytoplasm

► Nuclear division is usually followed by cytokinesis. Animal cell cytoplasm usually divides by a furrowing of the plasma membrane, caused by the contraction of cytoplasmic microfilaments. In plant cells, cytokinesis is accomplished by vesicle fusion and the synthesis of new cell wall material. Review Figure 9.10

Reproduction: Sexual and Asexual

► The cell cycle can repeat itself many times, forming a clone of genetically identical cells.

► Asexual reproduction produces a new organism that is genetically identical to the parent. Any genetic variety is the result of mutations.

► In sexual reproduction, two haploid gametes—one from each parent—unite in fertilization to form a genetically unique, diploid zygote. Review Figure 9.12

► In sexually reproducing organisms, certain cells in the adult undergo meiosis, a process by which a diploid cell produces haploid gametes. Each gamete contains a random mix of one of each pair of homologous chromosomes from the parent.

► The number, shapes, and sizes of the chromosomes constitute the karyotype of an organism. Review Figure 9.13

Meiosis: A Pair of Nuclear Divisions

► Meiosis reduces the chromosome number from diploid to haploid and ensures that each haploid cell contains one member of each chromosome pair. It consists of two nuclear divisions. Review Figure 9.14

► During prophase I of the first meiotic division, homologous chromosomes pair up with each other, and material may be exchanged by crossing over between nonsister chromatids of two adjacent homologs. In metaphase I, the paired homologs gather at the equatorial plate. Each chromosome has only one kinetochore and associates with polar microtubules for one

pole. In anaphase I, entire chromosomes, each with two chromatids, migrate to the poles. By the end of meiosis I, there are two nuclei, each with the haploid number of chromosomes with two sister chromatids. Review Figures 9.14, 9.16

► In meiosis II, the sister chromatids separate. No DNA replication precedes this division, which in other aspects is similar to mitosis. The result of meiosis is four cells, each with a haploid chromosome content. Review Figures 9.14, 9.17

► Both crossing over during prophase I and the random selection of which homolog of a pair migrates to which pole during anaphase I ensure that the genetic composition of each haploid gamete is different from that of the parent and from that of the other gametes. The more chromosome pairs there are in a diploid cell, the greater the diversity of chromosome combinations generated by meiosis.

Meiotic Errors

► In nondisjunction, one member of a homologous pair of chromosomes fails to separate from the other, and both go to the same pole. This event leads to one gamete with an extra chromosome and another other lacking that chromosome. Fertilization with a normal haploid gamete results in aneu-ploidy and genetic abnormalities that are invariably harmful or lethal to the organism. Review Figure 9.18

Cell Death

► Cells may die by necrosis or may self-destruct by apopto-sis, a genetically programmed series of events that includes the detachment of the cell from its neighbors and the fragmentation of its nuclear DNA. Review Figure 9.19

Applying Concepts

1. Compare chromatids and chromosomes. At what stages during mitosis and meiosis are chromatids present?
2. Strains of organisms unable to carry out certain functions in the cell cycle have been invaluable to scientists to determine what happens in the normal cell cycle. Describe the cell cycle in cells
 - a. lacking the G1 cyclin.
 - b. lacking the mitotic spindle.
 - c. lacking the microfilaments involved in plasma mem brane contraction.
3. Compare the sequence of events in the mitotic cell cycle with the sequence in programmed cell death.
4. The potato plant has 24 pairs of chromosomes. What is the number of
 - a. chromatids in a cell at prophase of mitosis?
 - b. chromosomes in a cell at anaphase of mitosis?
 - c. chromatids in a cell at metaphase I of meiosis?
 - d. chromatids in a cell at prophase II of meiosis?

10

Genetics: Mendel and Beyond

jx

17

s

In the middle eastern desert 1,800 years ago, a rabbi faced a serious dilemma. A Jewish woman had given birth to a son. As required by laws first set down by God's commandment to Abraham almost 2,000 years previously and reiterated later by Moses, the mother brought her 8-day-old son to the rabbi for ritual penile circumcision. The rabbi knew that the woman's two previous sons had bled to death when their foreskins were cut. Yet the Biblical commandment remained: Unless he was circumcised, the boy could not be counted among those with whom God had made His solemn covenant. After consultation with other rabbis, it was decided to exempt this, the third son.

Almost one thousand years later, in the twelfth century, the physician and biblical commentator Moses Maimonides reviewed this and numerous other cases in the rabbinical literature, and stated that in sudiinslancesjhe third son should not be circumcised. Furthermore, the ban should apply whether the son was "from her first husband or from her second husband." The bleeding disorder, he reasoned, was clearly carried by the mother and passed on to her sons.

Knowing nothing of our modern vision of genetics, these rabbis linked a human disease (which turns out to be hemophilia A) to a pattern of inheritance (which we know as sex linkage). Only in the past several decades have the precise biochemical

nature of hemophilia A and its genetic determination been worked out.

How do we account for, and predict, such patterns of inheritance? In this chapter, we will discuss how the units of inheritance, called genes, are transmitted from generation to generation of plants and animals, and show how many of the rules that govern genetics can be explained by the behavior of chromosomes during meiosis. We will also describe the interactions of genes with one another and with the environment, and the consequences of the fact that genes occupy specific positions on chromosomes.

An Ancient Ritual

A male infant undergoes ritual circumcision in accordance with Jewish laws. Sons of Jewish mothers who carry the gene for hemophilia may be exempt from the ritual.

The Foundations of Genetics

Much of the early study of biological inheritance was done with plants and animals of economic importance. Records show that people were deliberately cross-breeding date palm trees and horses as early as 5,000 years ago. By the early 1800s, plant breeding was widespread, especially with ornamental flowers such as tulips. Half a century later, in 1866, Gregor Mendel used the knowledge of plant reproduction to design and conduct experiments on inheritance. Although his published results were neglected by scientists for 40 years, they ultimately became the foundation for the science of genetics.

Plant breeders showed that both parents contribute equally to inheritance

Plants are easily grown in large quantities, many produce large numbers of offspring (in the form of seeds), and many have relatively short generation times. In most plant species, the same individuals have both male and female reproductive organs, permitting each plant to reproduce as a male, as a female, or as both. Best of all, it is often easy to control which individuals mate (Figure 10.1).



RESEARCH METHOD

Anatomy of a pea flower (shown in long section)



The stigma, where the pollen lands, is at the tip of the carpel.

Anthers at the tip of the stamen are the sites of pollen production.

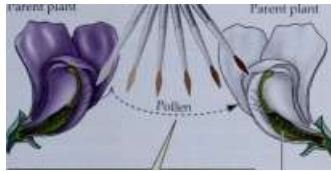
Stamens are the male sex organs.

The ovary is the female sex organ.

Pea flower cross-pollination

Parent plant

Parent plant

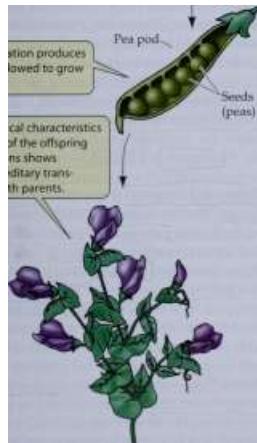


I Using a brush, pollen is transferred from anthers of a purple flower to the stigma of a white flower whose anthers have been snipped off.

I This cross-pollination produces 1 seeds that are allowed to grow r[^]s. into new plants.

I Analysis of physical characteristics (see Table 10.1) of the offspring over 2 generations shows evidence of hereditary transmission from both parents.

Pea pod



10.1 A Controlled Cross between Two Plants

Plants were widely used in early genetic studies because it is easy to control which individuals mate with which. Mendel used the pea plant, *Pisum sativum*, in many of his experiments.

GENETICS: MENDEL AND BEYOND 177

Some discoveries that Mendel found useful in his studies had been made in the late eighteenth century by a German botanist, Josef Gottlieb Kolreuter. Kolreuter studied the offspring of reciprocal crosses between plants and showed that the two parents contributed equally to the characteristics inherited by their offspring. In A reciprocal cross} plants are crossed with (mated with) each other in

opposite directions. For example, in one cross, males that have white flowers are mated with females that have red flowers, while in a complementary cross, red-flowered males and white-flowered females are the parents. In Kolreuter's experience, such reciprocal crosses always gave identical results.

Although the concept of equal parental contributions was an important discovery, the nature of what exactly the parents were contributing—the units of inheritance—remained unknown. Laws of inheritance proposed at the time favored the concept of blending. If a plant that had one form of a characteristic (say, red flowers) was crossed with one that had a different form of that characteristic (blue flowers), the offspring would be a blended combination of the two parents (purple flowers).

According to the blending concept, it was thought that once heritable elements were combined, they could not be separated again (like combined inks). The red and blue genetic determinants were thought to be forever blended into the new purple one. Then, about a century after Kolreuter completed his work, Mendel began his.

Mendel's discoveries were overlooked for decades

Gregor Mendel was an Austrian monk, not an academic scientist, but he was qualified to undertake scientific investigations. Although in 1850 he had failed an examination for a teaching certificate in natural science, he later undertook intensive studies in physics, chemistry, mathematics, and various aspects of biology at the University of Vienna. His work in physics and mathematics probably led him to apply experimental and quantitative methods to the study of heredity—and these were the key ingredients in his success.

Mendel worked out the basic principles of inheritance in plants over a period of about 9 years. His work culminated in a public lecture in 1865 and a detailed written account published in 1866. Mendel's paper appeared in a journal that was received by 120 libraries, and he sent reprinted copies (of which he had obtained 40) to several distinguished scholars. However, his theory was not accepted. In fact, it was ignored.

The chief difficulty was that the most prominent biologists of Mendel's time were not in the habit of thinking in mathematical terms, even the simple terms used by Mendel. Even Charles Darwin, whose theory of evolution by natural selection

depended on genetic variation among individuals, failed to understand the significance of Mendel's findings. In fact, Darwin performed breeding experiments like Mendel's on snapdragons and got data similar to Mendel's, but he missed the point, still relying on the concept of blending. In addition, Mendel had little credibility as a biologist; in-

178 CHAPTER TEN

deed, his lowest grades were in biology! Whatever the reasons, Mendel's pioneering paper had no discernible influence on the scientific world for more than 30 years.

Then, in 1900, Mendel's discoveries burst into prominence as a result of independent experiments by three plant geneticists: the Dutch Hugo de Vries, the German Karl Correns, and the Austrian Erich von Tschermak. Each of these scientists carried out crossing experiments and obtained quantitative data about the progeny; each published his principal findings in 1900; each cited Mendel's 1866 paper. By that time, meiosis had been observed and described. At last the time was ripe for biologists to appreciate the significance of what these four geneticists had discovered.

Mendel's Experiments and the Laws of Inheritance

That Mendel was able to make his discoveries before the discovery of meiosis was due in part to the methods of experimentation he used. Mendel's work is a fine example of preparation, execution, and interpretation. Let's see how he approached each of these steps.

Mendel devised a careful research plan

Mendel chose the garden pea for his studies because of its ease of cultivation, the feasibility of controlled pollination (see Figure 10.1), and the availability of varieties with differing traits. He controlled pollination, and thus fertilization, of his parent plants by manually moving pollen from

one plant to another. Thus he knew the parentage of the offspring in his experiments. If untouched, the pea plants Mendel studied naturally self-pollinate—that is, the female organ of each flower receives pollen from the male organs of the same flowers—and he made use of this natural phenomenon in some of his experiments.

Mendel began by examining different varieties of peas in a search for heritable characters and traits suitable for study. A character such as flower color; a pair of contrasting traits

one heritable character trait is one that is passed from parent to offspring. Mendel looked for (characters that had well-defined, contrasting alternative traits, such as purple flowers versus white flowers that were true-breeding.

To be considered true-breeding, the observed trait must be the dominant form present for many generations. In other words, peas with white flowers, when crossed with one another, would have to give rise only to progeny with white flowers for many generations; tall plants bred to tall plants would have to produce only tall progeny.

Mendel isolated each of his true-breeding strains by repeated inbreeding (done by crossing of sibling plants that were seemingly identical, or allowing individuals to self-pollinate) and selection. In most of his work, Mendel concentrated on the seven pairs of contrasting traits shown in Table 10.1. Before performing any given cross, he made sure that each potential parent was from a true-breeding strain—an essential point in his analysis of his experimental results.



In Experiment 1, Mendel studied the inheritance of seed shape. We know today that the wrinkled seeds possess an abnormal form of starch. Contrast their appearance with that of the spherical seeds below.

Mendel then collected pollen from one parental strain and placed it onto the stigma (female organ) of flowers of the other strain. The plants providing and receiving the pollen were the parental generation, designated P. In due course, seeds formed and were planted. The resulting new plants constituted the first filial generation, F_1 . Mendel and his assistants examined each F_1 plant to see which traits it bore and then recorded the number of F_1 plants expressing each trait. In some experiments the F_1 plants were allowed to self-pollinate and produce a second filial generation, or F_2 . Again, each F_2 plant was identified and counted.

In sum, Mendel devised a well-organized plan of research, pursued it faithfully and carefully, recorded great amounts of quantitative data, and analyzed the numbers he recorded to explain the relative proportions of the different kinds of progeny. His 1866 paper stands to this day as a model of clarity. His results and the conclusions to which they led are the subject of the next few sections.

Mendel's Experiment 1 examined a monohybrid cross

"Experiment 1" in Mendel's paper involved a monohybrid cross—one in which each parent pea plant was true-breeding for a given character, but in this case each displayed a different form of that character (a different trait). He took pollen from

plants of a true-breeding strain with wrinkled seeds and placed it on the stigmas of flowers of a true-breeding, spherical-seeded strain (Figure 10.2). He also performed the reciprocal cross, placing pollen from the spherical-seeded strain on the stigmas of flowers of the wrinkled-seeded strain.

In both cases, all the F₁ seeds that were produced were spherical—it was as if the wrinkled trait had disappeared completely. The following spring Mendel grew 253 F₁ T plants from these spherical seeds, each of which was allowed to self-pollinate—this was the monohybrid cross—to produce F₂ seeds. In all, there were 7,324 F₂ seeds, of which 5,474 were spherical and 1,850 wrinkled (Figure 10.3).

Mendel concluded that the spherical seed trait was dominant: In the F₁ generation, it was always expressed rather

GENETICS: MENDEL AND BEYOND 179

EXPERIMENT

Question: When two strains with contrasting traits breed, are their characteristics irreversibly blended in succeeding generations?

METHOD

Plant a

true-breeding spherical seed

P seeds

f

P plants are cross-pollinated

Plant a

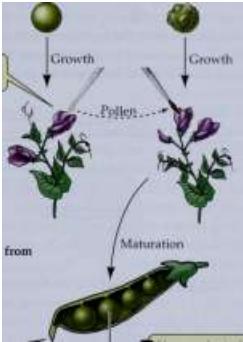
true-breeding wrinkled seed

Growth

P plants

F₁ seeds from P plant

all F₁ seeds are spherical; all F₁ T all spherical. J



Q Plant a spherical F₁ Seed

F₁ plant

Pollen

t



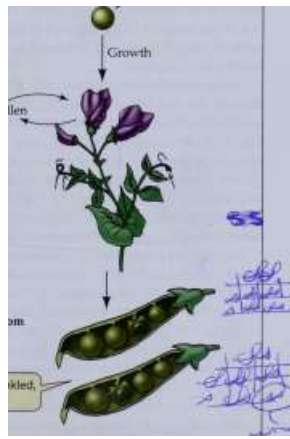
The F₁ plants self-pollinate.

RESULTS

F₂ seeds from F₁ plant

f

F₂ seeds: 1/4 are wrinkled 3/4 are spherical



Conclusion: There is no irreversible blending of characteristics. A trait can reappear in succeeding generations.

70.3 Mendel's Experiment 1

The pattern Mendel observed in the F₂ generation— $\frac{3}{4}$ of the seeds wrinkled, $\frac{3}{4}$ spherical—was the same no matter which variety contributed the pollen in the parental generation.

v^W

180 CHAPTER TEN

than the wrinkled seed trait, which he called recessive. In each of the other six pairs of traits Mendel studied, one proved to be dominant over the other. When he crossed plants differing in one of these traits, only one of each pair of traits was evident in the F₁ generation. However, the trait that was not seen reappeared in the F₂.

Of most importance, the ratio of the two traits in the F₂ generation was always the same—approximately $\frac{3}{4}$. That is, three-fourths of the F₂ showed the dominant trait and one-fourth showed the recessive trait (see Table 10.1). In Mendel's Experiment 1, the ratio was $\frac{5,474}{1,850} = 2.96:1$. The reciprocal cross in the parental generation gave a similar outcome in the F₂.

By themselves, the results from Experiment 1 disproved the widely held belief that inheritance is always a blending phenomenon. According to the blending theory, Mendel's F₁ seeds should have had an appearance intermediate between those of the two parents—in other words, they should have been slightly wrinkled. Furthermore, the blending theory offered no explanation for the reappearance of the wrinkled trait in the F₂ seeds after its apparent absence in the F₁ seeds.

Mendel proposed that the units responsible for the inheritance of specific traits are present as discrete particles that occur in pairs and segregate (separate) from one another during the formation of gametes. According to this theory, the units of inheritance retain their identity in the presence of other units. The particulate theory is a sharp contrast to the concept of blending, in which the units of inheritance were believed to lose their identities when mixed together.

As he wrestled mathematically with his data, Mendel reached the conclusion that each pea plant has two units of inheritance for each character, one from each parent. During the production of gametes, only one of these paired units for a given character is given to a gamete. Hence each gamete contains one unit, and the resulting zygote contains two, because it was produced by the fusion of two gametes. This conclusion is the core of Mendel's model of inheritance. Mendel's unit of inheritance is now called a gene.



Mendel reasoned that in Experiment 1, the spherical-seeded parent had a pair of genes of the same type, which we will call S, and the parent with wrinkled seeds had two s genes. The SS parent produced gametes each containing a single S, and the ss parent produced gametes each with a single s. Each member of the F₁ generation had an S from one parent and an s from the other; an F₁ could thus be described as Ss. We say that S is dominant over s because the s trait is not evident when both forms of the gene are present.

The different forms of a gene (S and s in this case) are called alleles. Individuals that are truebreeding for a trait contain two copies of the same allele. For example, all the individuals in a population of a strain of true-breeding peas with wrinkled seeds must have the allele pair ss; if S were present, the plants would produce spherical seeds.

We say that the individuals that produce wrinkled seeds are homozygous for the alleles, meaning that they have

<h





two copies of the same allele (ss). Some peas with spherical seeds—the ones with the genotype SS—are also homozygous. However, not all plants with spherical seeds have the SS genotype. Some spherical-seeded plants, like Mendel's F_1 are heterozygous: They have two different alleles of the gene in question (In this case, Ss).

To illustrate these terms with a more complex example, one in which there are three gene pairs, an individual with the genotype AABbcc is homozygous for the A and C genes—because it has two A alleles and two c alleles—but heterozygous for the B gene because it contains the B and b alleles. An individual that is homozygous for a character is sometimes called a homozygote; a heterozygote is heterozygous for the character in question.

The physical appearance of an organism is its phenotype. Mendel correctly supposed the phenotype to be the result of the genotype, or genetic constitution, of the organism showing the phenotype. In Experiment 1 we are dealing with two phenotypes (spherical-seeded and wrinkled-seeded). As we will see in the next section, the F_2 generation contains these two phenotypes and three genotypes. The wrinkled-seed phenotype is produced only by the genotype ss, whereas the spherical-seed phenotype may be produced by the genotypes SS or Ss.

Mendel's first law says that alleles segregate

How does Mendel's model of inheritance explain the composition of the F_2 generation in Experiment 1? Consider first the F_1 which has the spherical-seeded phenotype and the Ss genotype. According to Mendel's model when any individual produces gametes, the alleles separate, so that each gamete receives only one member of the pair of alleles. This is Mendel's first law, the law of segregation. In Experiment 1, half the gametes produced by the F_1 contained the S allele and half the s allele.

During self-pollination, the random combination of gametes produces the F_2 generation (Figure 10.4). Three different F_2 genotypes are possible: SS, Ss (which is the same thing as sS), and ss. Our quantitative way of looking at things may lead us to wonder what proportions of these genotypes we might expect to observe in the F_2 progeny. The expected frequencies of these three genotypes may be determined by using the Punnett square, devised in 1905 by the British geneticist Reginald Crundall Punnett.

The Punnett square is a device that reminds us to consider all possible combinations of gametes. The square looks like this:

Female c c Male

gametes S

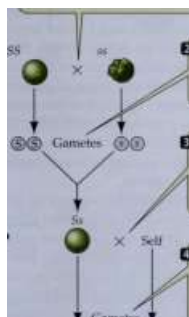


It is a simple grid with all possible sperm genotypes shown across one side and all possible egg genotypes along another side. To complete the grid, we fill in each square with the corresponding sperm genotype and egg genotype, giving

Q A parent homozygous for the allele for spherical seeds is crossed with a parent homozygous for the allele for wrinkled seeds.

Parental (P) generation

F_1 generation



Each homozygous parent makes haploid gametes of one kind, genotype S or genotype s.

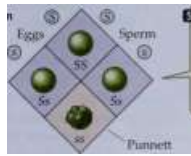
The parental gametes combine to produce F_1 plants with the Ss genotype and a spherical seed phenotype.

Gametes

© (D) ©

The heterozygous F_1 plant makes two kinds of haploid gametes, genotype S and genotype s.

F_2 generation



When F₁ plants self-pollinate, the S and s gametes combine randomly to produce two different seed phenotypes in the F₂ plants, as this Punnett square shows.

Punnett square

10.4 Mendel's Explanation of Experiment 1

Mendel concluded that inheritance depends on factors from each parent, and that these factors are discrete units that do not blend in the offspring.

Using the diploid genotype of one member of the F₂ generation. For example, to fill the rightmost square, we put in the S from the egg (female gamete) and the s from the sperm (male gamete), yielding Ss.

Examination of the Punnett square in Figure 10.4 reveals that self-pollination of the F₁ genotype Ss will give the three F₂ genotypes in the expected ratio 1 SS:2 Ss:1 ss. Because S is dominant and s recessive, only two phenotypes result, in the ratio of 3 spherical (SS and Ss) to 1 wrinkled (ss), just as Mendel observed.

Mendel did not live to see his theory placed on a sound physical footing based on chromosomes and DNA. Genes are now known to be regions of the DNA molecules in chromosomes. More specifically, a gene is a portion of the DNA that resides at a particular position, called a locus (plural loci), within the chromosome and that encodes a particular function. Mendel arrived at his law of segregation with no knowledge of chromosomes or meiosis, but today we can picture the different alleles of a gene segregating as chromosomes separate in meiosis I (Figure 10.5).

Mendel verified his hypothesis by performing a test cross.

The test cross is a way to test whether a given individual showing a dominant trait is homozygous or heterozygous. In a test cross, the individual in question is crossed with an individual known to have the recessive trait—an easy individual to identify, because in order to have the recessive phenotype it must be homozygous for the recessive trait.

For the pea gene that we have been considering, the recessive homozygote used for the test cross is ss. The individual being tested may be described initially as S- because

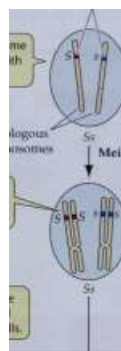
Alleles of gene for seed shape

This site on the chromosome is the locus of the gene with the alleles S and s.

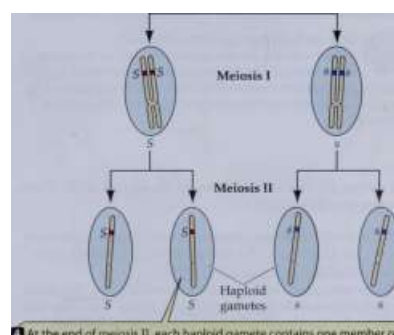
Homologous chromosomes

Before meiosis I, each of the homologous chromosomes replicates.

At the end of meiosis I, the two alleles are segregated into separate daughter cells.



Meiotic interphase



I At the end of meiosis II, each haploid gamete contains one member of each pair of homologous chromosomes, and thus one allele for each pair of genes.

70.5 Meiosis Accounts for the Segregation of Alleles

Although Mendel had no knowledge of chromosomes or meiosis, we now know that a pair of alleles resides on homologous chromosomes, and that meiosis segregates those alleles.

182 CHAPTER TEN

EXPERIMENT

Question: How can we determine if an organism expressing a dominant phenotype is homozygous or heterozygous for the alleles determining that phenotype?

Spherical peas are of undetermined genotype.



METHOD

ss

Wrinkled peas have known genotype (homozygous recessive).

X

m

If the plant being tested is homozygous:

SS

ss

If the plant being tested is heterozygous:

Ss SS

3 x #

o o Gametes © © © ©

© ©

RESULTS

© ©

Egg s /" ^ Sperm Eggs / ^ ^ Sperm

) V ^ © © S* ©

Ss q Ss

Ss

All progeny show the dominant phenotype.

Conclusion: The plant being tested must be homozygous.

55 •

Ss ►

Half the seeds from the cross are wrinkled, and half are spherical.

Conclusion: The plant being tested must be heterozygous.



70.6 Homozygous or Heterozygous?

A plant with a dominant phenotype may be homozygous or heterozygous. Its genotype can be determined by making a test

cross, which involves crossing it with a homozygous recessive plant and observing the phenotypes of the progeny produced. we do not yet know the identity of the second allele. There are two possible results:

- If the individual being tested is homozygous dominant (SS), all offspring of the test cross will be Ss and show the dominant trait (spherical seeds).
- If the individual being tested is heterozygous (Ss), then approximately half of the offspring of the test cross will show the dominant trait (Ss), but the other half will be homozygous for, and will show, the recessive trait (ss) (Figure 10.6).

These were exactly the results that Mendel obtained; thus Mendel's model accurately predicts the results of such test crosses.

Mendel's second law says that alleles of different genes assort independently

What happens if two parents that differ at two or more loci are crossed? Consider an organism heterozygous for two genes, Ss and Yy, in which S and Y came from its mother and s and y came from its father. When this organism makes gametes, do the alleles of maternal origin (S and Y) go together to one gamete and those of paternal origin (s and y) to another gamete? Or can a single gamete receive one maternal and one paternal allele, S and y (or T and c)? To answer these questions Mendel performed a series of dihybrid crosses made between parents that are identical double heterozygotes.

In these experiments, Mendel began with peas that differed for two characters of the seeds: seed shape and seed color. One true-breeding strain produced only spherical, yellow seeds (SSYY) and the other strain produced only wrinkled, green ones (ssyy). A cross between these two strains produced an F₁ generation in which all the plants were SsYy. Because the S and Y alleles are dominant, these F₁ seeds were all yellow and spherical.

Mendel continued this experiment to the next generation—the dihybrid cross. There are two ways in which these doubly heterozygous plants might produce gametes, as Mendel saw it. (Remember that he had never heard of chromosomes or meiosis.)

First, if the alleles maintain the associations they had in the original parents (that is, if they are linked), then the F₁ plants should produce two types of gametes (SY and sy), and the F₂ progeny resulting from self-pollination of the F₁ plants should consist of three times as many plants bearing spherical, yellow seeds as ones with wrinkled, green seeds. Were such results to be obtained, there might be no reason to suppose that seed shape and seed color were regulated by two different genes, because spherical seeds would always be yellow, and wrinkled seeds would always be green.

The second possibility is that the segregation of S from s is independent of segregation of Y from y during the production of gametes (that is, that they are unlinked). In this case, four kinds of gametes should be produced, in equal numbers: SY, Sy, sY, and sy. When these gametes combine at random, they should produce an F₂ of nine different genotypes. The progeny could have any of three possible genotypes for shape (SS, Ss, or ss) and any of three possible genotypes for color (YY, Yy, or yy). The combined nine genotypes should produce just four phenotypes (spherical yellow, spherical green, wrinkled yellow, wrinkled green). By using a Punnett square, we can show that these four phenotypes would be expected to occur in a ratio of 9:3:3:1. (Figure 10.7).

Mendel's dihybrid crosses produced the results predicted by the second possibility. Four different phenotypes appeared in the F₂ in a ratio of about 9:3:3:1. The parental traits appeared in new combinations of the phenotypic classes (spherical green and wrinkled yellow). Such new combinations are called recombinant phenotypes.

Parental

(P) generation



X



SsYy plants make four kinds of gametes in equal proportions.

F₁ generation

Gametes



The gametes combine randomly to produce an F₂ generation with four phenotypes in a 9:3:3:1 ratio.

Sy) Sperm



Q @ Q



70.7 Independent Assortment

The 16 possible combinations of gametes result in 9 different genotypes. Because S and Y are dominant over s and y, respectively, the 9 genotypes determine 4 phenotypes in the ratio of 9:3:3:1.

These results led Mendel to the formulation of what is now known as Mendel's second law of independent assortment. This law of independent assortment is not as universal as the law of segregation, because it applies to genes that lie on separate chromosomes but not necessarily to those that lie on the same chromosome. However, it is correct to say that chromosomes segregate independently during the formation of gametes, and so do any two genes on separate chromosome pairs (Figure 10.8).

Punnett squares or probability calculations: A choice of methods

Many people find it easiest to solve genetics problems using probability calculations, perhaps because the principle is familiar. When we flip a coin, for example, we expect that it has an equal probability of landing "heads" or "tails." For a given toss of a fair coin, the probability of heads is independent of what happened in all the previous tosses. A run of ten straight heads implies nothing about

GENETICS: MENDEL AND BEYOND 183

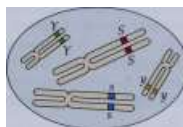
the next toss. No "law of averages" increases the likelihood that the next toss will come up tails, and no "momentum" makes an eleventh occurrence of heads any more likely. On the eleventh toss, the odds are still 50:50.

The basic conventions of probability are simple: If an event is absolutely certain to happen, its probability is 1. If it cannot happen, its probability is 0. Otherwise, its probability lies between 0 and 1. A coin toss results in heads approximately half the time, and the probability of heads is $1/2$ —as is the probability of tails.

multiplying probabilities. If two coins (a penny and a dime, say) are tossed, each acts independently of the other. What, then, is the probability of both coins coming up heads? Half the time, the penny comes up heads; of that fraction, half the time the dime also comes up heads. Therefore, the joint probability of two heads is half of one-half, or $1/2 \times 1/2 = 1/4$. To find the joint probability of independent events, then, the

Diploid parent

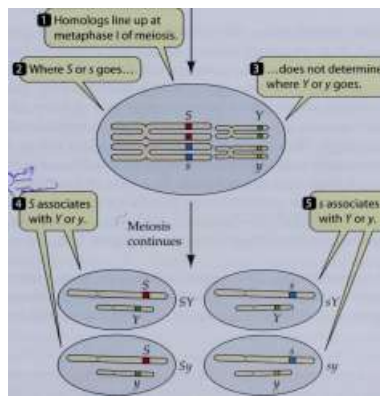
SsYy



Homologs line up at metaphase I of meiosis

Where does... j

-cr*



<-§? O S associates with Vory.

Four haploid gametes

SY, Sy, sY, sy

rn



70.8 Meiosis Accounts for Independent Assortment of Alleles

We now know that alleles are segregated independently during metaphase I of meiosis. Thus a parent of genotype SsYy can form gametes with four different genotypes; which ones actually form is a matter of chance.

184 CHAPTER TEN

70.9 Joint Probabilities of Independent Events

Like two tosses of a coin, the segregation of each allele into a sperm or an egg is an independent event. The probability of any given combination of alleles from a sperm and an egg is obtained by multiplying the probabilities of each event; this is the probability of producing a homozygote. Since a heterozygote can be formed in two ways, the two probabilities are added together.

t) Each individual outcome is the result of two independent events, each with a probability of $1/2$; the joint probability is $V_2 \times 1/2 = V_4$ (multiplication rule)

I Two coin tosses are individual events.

/

general rule is to multiply the probabilities of the individual events (Figure 10.9).

the monohybrid cross. To apply a probabilistic approach to genetics problems, we need only deal with gamete formation and random fertilization instead of coin tosses. A homozygote can produce only one type of gamete, so, for example, an SS individual has a probability equal to 1 of producing gametes with the genotype S. The heterozygote Ss produces S gametes with a probability of V_2 , and

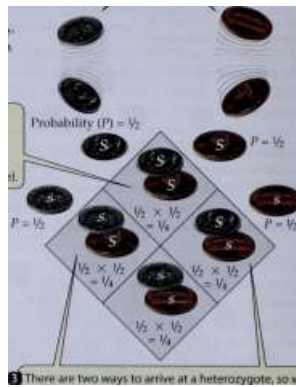
y s gametes with a probability of V_2 .

-Consider the F₂ progeny of the cross in Figure 10.4. J^hey are obtained by self-pollination of F₁ plants of geno-

.>type Ss. The probability that an F₂ plant will have the genotype SS must be $V_2 \times V_2 = 1/4$ because there is a 50:50 chance that the sperm will have the genotype S, and this chance is independent of the 50:50 chance that the egg will have the genotype S. Similarly, the probability of ss offspring is $V_2 \times V_2 = 1/4$.

adding probabilities. The probability of an F₂ plant getting S from the sperm and s from the egg is also $1/4$, but remember that the same genotype can also result from s in the sperm and S in the egg, with a probability of $1/4$. The probability of an event that can occur in two or more different ways is the sum of the individual probabilities of those ways. Thus the probability that an F₂ plant will be a heterozygote is equal to the sum of the probabilities of each way of forming a heterozygote: $1/4 + 1/4 = 1/2$ (see Figure 10.9). The three genotypes are therefore expected in the ratio 1/4 SS: 1/2 Ss: 1/4 ss—hence the 1:2:1 ratio of genotypes and the 3:1 ratio of phenotypes seen in Figure 10.4.





There are two ways to arrive at a heterozygote, so we add the probabilities of the two individual outcomes: $1/4 + 1/4 = 1/2$ (addition rule).

phenotypes are easily determined by probabilities. Let's see how this works for the experiment shown in Figure 10.7.

The probability that a seed will be spherical is $3/4$, as we have just seen. By the same reasoning, the probability that a seed will be yellow is also $3/4$. The two characters are determined by separate genes and are independent of each other, so the joint probability that a seed will be both spherical and yellow is $3/4 \times 3/4 = 9/16$. For the wrinkled, yellow members of the F_2 generation, the probability of being yellow is again $3/4$; the probability of being wrinkled is $1/2 \times 1/2 = 1/4$. The joint probability that a seed will be both wrinkled and yellow, then, is $3/4 \times 1/4 = 3/16$. The same probability applies, for similar reasons, to the spherical, green F_2 seeds. Finally, the probability that F_2 seeds will be both wrinkled and green must be $1/4 \times 1/4 = 1/16$. Looking at all four phenotypes, we see they are expected in the ratio of $9:3:3:1$.

the dihybrid cross. If F_1 plants heterozygous for two independent characters self-pollinate, the resulting F_2 plants express four different phenotypes. The proportions of these

Generation I

Generation II

Generation III

*S

cm

Parents

M

Oldest

Youngest

10.10 Pedigree Analysis and Dominant Inheritance

A human pedigree showing dominant inheritance. This family carries the allele for Huntington's disease. Everyone who inherits this allele is affected.



iteo *5i*a *£i

Every affected individual has an affected parent.

About $1/2$ of the offspring (of both y^+ -i sexes) are affected.

Siblings

T, ~Z, : ^

Male ^

70.7 7 Recessive Inheritance This family carries the allele for albinism, a recessive trait. In an affected individual, the trait must be inherited from two heterozygous parents or (rarely) from one homozygous and one heterozygous parent. In this case the heterozygous parents are cousins, but the same result could occur -':-e c-='r-:= .'e-e --'•= ='~z z .: -e:e-z. ;: .-■

Probability calculations and Punnett squares give the same results. Learn to do genetics problems both ways, and then decide which method you prefer.

Mendel's laws can be observed in human pedigrees

A few years after Mendel's work was uncovered by plant breeders, Mendelian inheritance was found in humans. By now, patterns of over 2,500 inherited human characteristics have been discovered.

Mendel worked out the rules of inheritance by performing many planned crosses and counting many offspring. Neither of these approaches is possible with humans. So human geneticists rely on pedigrees, family trees that show the segregation of phenotypes (and alleles) in several generations of related individuals.

Because human pedigrees do not show the exact proportions of offspring that Mendel saw in his pea plants (see Table 10.1). For example, when two heterozygous people (Aa) have children, there is a 25 percent probability that the child will be homozygous recessive (aa). Over many such marriages, one-fourth of all the children will be homozygous recessive (aa). But what about a single marriage? In human families, while the odds for each child remain the same, the results are often different. So, in a family with two children, both could easily be aa (or Aa or AA).

To deal with this ambiguity, human geneticists assume that any allele that is rare in the population. This means that in a given family with the rare allele (say, one parent is Aa), it is highly unlikely that an outsider marrying into the family will have the same rare allele (the outsider is most likely AA).

Human geneticists may wish to know whether a particular rare allele is dominant or recessive. Figure 10.10 depicts a pedigree showing the pattern of inheritance of a rare dominant phenotype. The following are the key features to look for in such a pedigree:

- ▶ Every affected person has an affected parent
- ▶ About half of the offspring of an affected person are also affected.
- ▶ The phenotype occurs equally in both sexes.

Heterozygous for GENETICS: MENDEL AND BEYOND 185

interest (inferred)

relatives are

Compare this pattern with Figure 10.11, which shows the pattern of inheritance of a rare recessive phenotype:

- ▶ Affected people usually have parents who are both not affected.
- ▶ About one-quarter of the children of unaffected parents can be affected.
- ▶ The phenotype occurs equally in both sexes.

In pedigrees showing recessive inheritance, it is not uncommon to find a marriage of two relatives. This observation is a result of the rarity of phenotypically abnormal alleles. For two phenotypically normal parents to have an affected child (aa), the parents must both be heterozygous (Aa). If the allele is rare in the general population, the chance of two people marrying who are both carrying the same rare allele is quite low. On the other hand, if the particular recessive allele is present in a family, two cousins might share it (see Figure 10.10). This is why studies on populations isolated either culturally (by religion, as with the Amish in the United States) or geographically (as on islands) have been so valuable to human geneticists. People in these groups tend either to have large families, or marry among themselves, or both.

Because the major use of pedigree analysis is the clinical evaluation and counseling of patients with inherited abnormalities, a single pair of alleles is usually followed. However, just as pedigree analysis shows the segregation of alleles, it also can show independent assortment if two different allele pairs are considered.

Alleles and Their Interactions

Let's move on to the extensions of Mendelian genetics that have been developed by other researchers, mostly in the early part of the twentieth century. Decades after Mendel's work, others discovered that his hereditary particles—genes—are chemical entities—DNA sequences—that are

186 CHAPTER TEN

Possible genotypes

Phenotype

CC, Cc, cc

Dark gray

Light gray

Genotype

Chinchilla

cc

Light gray

Himalayan

Albino

Win* ejtz

s

usually expressed as proteins. Accordingly, the different alleles of a gene at the same locus, which result in slightly different protein products. This chapter we'll see the molecular basis of the distinctions between alleles. In this section we deal with how alleles relate to one another, some of their general properties, and how they arise.

In many cases, alleles do not show simple relationships between dominance and recessiveness. In others, a single allele may have multiple phenotypic effects when it is expressed. Existing alleles can form new alleles by mutation, so there can be many alleles for a single character.

New alleles arise by mutation

Different alleles exist because any gene is subject to mutation, which occurs when a gene is changed to a stable, heritable new form. In other words, an allele can mutate to become a different allele. Mutation, which will be discussed in detail in Chapter 12, is a random process; different copies of the same gene may be changed in different ways, depending on how and where the DNA sequence changes.

One particular allele of a gene may be defined as the wild type or standard, because it is present in most individuals. Its nature and gives rise to an expected trait or phenotype. Other alleles of that same gene, often called mutant alleles, may produce a different phenotype. The wild-type and mutant alleles reside at the same locus and are inherited according to the rules set forth by Mendel. A genetic locus with a wild-type allele that is present less than 99 percent of the time (the rest of the time)

to be polymorphic (from the Greek poly, "many," and morph, "form").

Many genes have multiple alleles

Because of random mutations, a group of individuals may have more than two alleles of a given gene. (Any one individual has only two alleles, of course—one from its mother and one from its father.) In fact, there are many examples of such multiple alleles.

Coat color in rabbits is determined by one gene with four alleles. There is a dominance hierarchy in the gene combinations:

$C > c^d > c^h > c$

Any rabbit with the C -allele (along with any of the four) is gray and a rabbit that is cc is albino. The intermediate col-

10.12 Inheritance of Coat Color in Rabbits

There are four alleles of the gene for coat color in rabbits. Different combinations of two alleles give different colors.

Colors result from the different allelic combinations shown in Figure 10.12.

Multiple alleles increase the number of possible phenotypes. In Mendel's monohybrid cross, there was just one pair of alleles (Ss) and two possible phenotypes (resulting from SS or Ss and ss). The four alleles of the rabbit coat

color gene produce five phenotypes.

Dominance is usually not complete

X In the single-pair alleles studied by Mendel, dominance is

complete when an individual is heterozygous. That is, an Ss

individual will express the S phenotype. However, many

genes have alleles, that are not dominant or recessive to one

another. Instead, the heterozygotes show an intermediate

phenotype—at first glance like that predicted by the old

blending theory of inheritance. For example, if a true-breed-

A true-breeding red snapdragon is crossed with a true-breeding white

One, all the F₁ flowers are pink. That this phenomenon can

not still be explained in terms of Mendelian genetics, rather than

blending, is readily demonstrated by a further cross.

According to the blending theory, if one of the pink F₁ snapdragons is crossed with a true-breeding white one, all the offspring should be a still lighter pink. In fact approximately 1/2 of the offspring are white, and the other half are the same shade of pink as the original F₁. When the F₁ pink snapdragons are allowed to self-pollinate, the resulting F₂ plants are distributed in a ratio of 1 red:2 pink:1 white (Figure 10.13). Clearly the hereditary particles—the genes—have not blended; they are readily sorted out in the F₂.

We can understand these results in terms of the Mendelian model. When a heterozygous phenotype is intermediate, as in the snapdragon example, the gene is said to be governed by incomplete dominance. All we need to do in cases like this is recognize that the heterozygotes show a phenotype intermediate between those of the two homozygotes.

We can also understand incomplete dominance in molecular terms. Remember that genes code for specific proteins, many of which are enzymes. Different alleles at a locus code for alternative forms of a protein. When the protein is an enzyme, the different forms often have different degrees of catalytic activity. In the snapdragon example, one allele codes for an enzyme that catalyzes a reaction leading to the forma-

Parental (P) generation

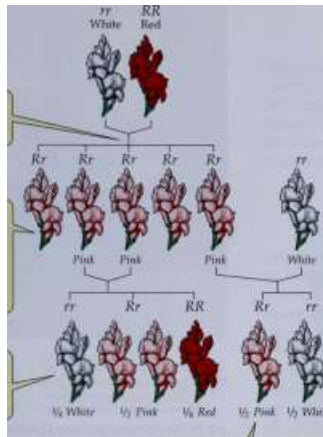
Q When true-breeding red and white parents cross, all plants in the F₁ generation are pink.

F₁ generation

Q Heterozygous snapdragons produce pink flowers—an intermediate phenotype—because the allele for red flowers is incompletely dominant over the allele for white ones.

F₂ generation

| When F₁ plants self-pollinate, they produce F₂ offspring that are white, pink, and red in a ratio of 1:2:1.



f

10.13 Incomplete Dominance Follows Mendel's Laws

An intermediate phenotype can occur in heterozygotes when neither allele is dominant. The phenotype (here, pink flowers) may give the appearance of a blended trait, but dominant and recessive traits reappear in their original forms in succeeding generations, as predicted by Mendel's laws.

tion of a red pigment in the flowers. The alternative allele codes for an altered enzyme that lacks catalytic activity for pigment production. Plants homozygous for this alternative allele cannot synthesize red pigment, and their flowers are white. Heterozygous plants, with only one allele for the functional enzyme, produce just enough red pigment that their flowers are pink.

There are more examples of incomplete dominance than of complete dominance in nature. Thus an unusual feature

A test cross confirms that pink snapdragons are heterozygous.

GENETICS: MENDEL AND BEYOND 187

In codominance, both alleles are expressed

Sometimes two alleles at a locus produce two different phenotypes that both appear in heterozygotes. An example of this phenomenon, called codominance, is seen in the ABO blood group system in humans.

Early attempts at blood transfusion—made before blood types were understood—frequently killed the patient. Around 1900, however, the Austrian scientist Karl Landsteiner mixed blood cells and serum (blood from which cells have been removed) from different individuals. He found that only certain combinations of blood are compatible. In other combinations, the red blood cells of one individual form clumps because of the presence in the other individual's serum of specific proteins, called antibodies, that react with foreign, or "nonself," cells. Proteins on nonself cells, called antigens, prompt the synthesis of antibodies. This discovery led to our ability to administer compatible blood transfusions that do not kill the recipient. Blood compatibility is determined by a set of three alleles (I^A, I^B, and i^o) at one locus, which determines certain proteins (antigens) on the surface of red blood cells. Different combinations of these alleles in different people produce four different blood types, or phenotypes: A, B, AB, and O (Figure 10.14).

Some alleles have multiple phenotypic effects

When a single allele has more than one distinguishable phenotypic effect, we say that the allele is ^

of Mendel's report is that all of the examples he described (see Table 10.1) are characterized by complete dominance. For dominance to be complete, a single copy of the dominant allele must produce enough of its protein product to give the maximum phenotypic response. For example, just one copy of the dominant allele T at one of the loci studied by Mendel leads to the production of enough of a growth-promoting chemical that the heterozygotes are as tall as homozygous dominant plants (TT)—the second copy of T causes no further growth of the stem. Homozygous recessive plants (tt) are much shorter because the allele t does not lead to the production of the growth promoter.

Blood type of cells

Genotype

Antibodies made by body

I^AI^A or I^Ai^o Anti-B

I^T or I^oI^o Anti-A

Reaction to added antibodies

Anti-A Anti-B

* * \$ ' ! ! !

■ A

Red blood cells that do not react with antibody remain evenly dispersed.



AB r

^ &

i a p

i^oi^o

Neither anti-A nor anti-B

Both

anti-A and anti-B

*

* 4

Red blood cells that react with antibody clump

& \$ — ^ m / ^ \ together (speckled

* «

appearance).



Cells of blood types A, B, AB, and O were mixed with anti-A or anti-B antibodies. As you look down the columns, note that each of the types, when mixed separately with anti-A and with anti-B, gives a unique pair of results; this is the basic method by which blood is typed. A person with type O blood is a good blood donor because O cells do not provoke or react with either anti-A or anti-B antibodies. A person with type AB blood is a good recipient, since neither type of antibody is made.

Ky

188 CHAPTER TEN

familiar example Of pleiotropy involves the allele responsible for the coloration pattern (light body, darker extremities) of Siamese cats, discussed later in this chapter. The same allele is also responsible for the characteristic crossed eyes of Siamese cats. Although these effects appear to be unrelated, both result from the same protein produced under the influence of the allele.

Gene Interactions

Thus far we have treated the phenotype of an organism, with respect to a given character, as a simple result of its genotype, and we have implied that a single trait results from the alleles of a single gene. In fact, several genes may interact - to determine a trait's phenotype. For example, height in people is determined by the actions of many genes, such as those that determine bone growth, hormone concentrations, and other aspects of development. Sometimes several genes act additively, so that the phenotype can be predicted by how many of these genes are active. To complicate things further, the physical environment may interact with the genetic constitution of an individual in determining the phenotype. Height in people, for example, is not determined only by their genes. Nutrition is just one environmental factor that undoubtedly has a strong influence on height.

Some genes alter the effects of other genes

Epistasis occurs when the phenotypic expression of one gene is affected by another gene. For example, several genes determine coat color in mice. The wild-type color is agouti, a grayish pattern resulting from bands on the individual hairs. The dominant allele *B* determines that the hairs will have bands and thus that the color will be agouti, whereas the homozygous recessive genotype *bb* results in non-banded hairs. On another chromosome, a second locus affects an early step in the formation of hair pigments. The dominant allele *A* at this locus allows normal color development, but *aa* blocks all pigment production. Thus, *aa* mice are all-white albinos, irrespective of their genotype at the *B* locus (Figure 10.15).

If a mouse with genotype *AABB* (and thus the agouti phenotype) is crossed with an albino of genotype *aabb*, the *F₁* is *AaBb* and has the agouti phenotype. If the *F₁* mice are crossed with each other to produce an *F₂* generation, then epistasis will result in an expected phenotypic ratio of 9 agouti:3 black:4 albino. (Can you show why? The underlying ratio is the usual 9:3:3:1 for a dihybrid cross with unlinked genes, but watch out for epistasis.)

In another form of epistasis, two genes are mutually dependent: The expression of each depends on the alleles of the other. The epistatic action of such complementary genes may be explained as follows: Suppose gene *A* codes for enzyme A in the metabolic pathway for purple pigment in flowers, and gene *B* codes for enzyme B:

Mice with genotype *aa* are albino regardless of their genotype for the other locus, because the *aa* genotype blocks all pigment production.



Mice with *bb* genotypes are black unless they are also *aa* (which makes them albino).

Mice that have at least one dominant allele at each locus are agouti.

colorless precursor

enzyme A

colorless intermediate

enzyme B

purple pigment

10.15 Genes May Interact Epistatically

Epistasis occurs when one gene alters the phenotypic effect of another gene. In these mice, the presence of the recessive genotype (*aa*) at one locus blocks pigment production, producing an albino mouse no matter what the genotype is at the second locus.

In order for the pigment to be produced, reactions must take place. The recessive alleles *a* and *b* code for nonfunctional enzymes. If a plant is homozygous for either *a* or *b*, the corresponding reaction will not occur, no purple pigment will form, and the flowers will be white.

Hybrid vigor results from new gene combinations and interactions

If Mendel's paper was the most important event in genetics in the nineteenth century, perhaps an equally important paper in applied genetics was published early in the twentieth century by G. H. Shull, entitled "The composition of a field of maize". For centuries, it has been known that if one takes two pure, homozygous genetic strains of a plant or animal, and crosses them, the result is offspring that are phenotypically much stronger, larger, and hence more "vigorous" than either of the parents (Figure 10.16).

Conversely, avoidance of inbreeding (mating between close relatives) is a time-honored tradition among farmers growing crops and in human societies (where it is called incest). The reason for this is that some of the alleles, some of which may be harmful, as we saw in our discussion of human pedigrees above.

Shull crossed two of the thousands of existing varieties of corn (maize). Both varieties produced about 20 bushels of corn per acre. But when he crossed them, the yield of their offspring was an astonishing 80 bushels per acre. This phenomenon is known as heterosis (short for heterozygosis), or hybrid vigor. The cultivation of hybrid corn spread rapidly in the United States and all over the world, quadrupling grain production. The practice of hybridization has spread to many other crops and animals used in agriculture.



Parent Parent Hybrid offspring

10.16 Hybrid Vigor in Corn

The heterozygous F₂ offspring is larger and stronger than either homozygous parent.

The actual mechanism by which hybrid vigor works is not known. A widely accepted hypothesis is over dominance, a situation in which the heterozygous condition in certain important genes is superior to either homozygote.

Polygenes mediate quantitative inheritance

Individual heritable characters are often found to be con-

trolled by groups of 'several genes, called polygenes, of which each allele intensifies or diminishes the observed

phenotype. As a result, variation in such characters is continuous, or quantitative) rather than, as in the examples we have been considering, discontinuous (or discrete). Many characters that vary continuously—such as height and other aspects of size, or skin color—are under polygenic control. The polygenes affecting a particular quantitative character are commonly located on many different chromosomes.

Humans differ with respect to the amount of a dark pigment, melanin, in their skin (Figure 10.17). There is great variation in the amount of melanin among different people, but much of this variation is determined by alleles at many loci. No alleles at these loci demonstrate dominance. Of course, skin color is not entirely determined by the genotype, since exposure to sunlight in light-skinned people can cause the production of more melanin (that is, a suntan).

The environment affects gene action

The phenotype of an individual does not result from its genotype alone. Genotype and environment interact to determine the phenotype of an organism. Environmental variables such as light, temperature, and nutrition can affect the translation of a genotype into a phenotype. A familiar example involves the Siamese cat. This handsome animal normally has dark points on its ears, nose, paws, and tail, although the rest of its body is white. These darkened extremities normally have a lower temperature than the rest of the body.

A few simple experiments show that the Siamese cat has a genotype that results in dark fur, but only at temperatures below the general body temperature. If some dark fur is removed from the tail and the cat is kept at higher than usual

AaBbCc AaBbCc

1/ 6/ 15/ 20/ 15/ 6/

/64 / (A' (A' (A' (A' (A'

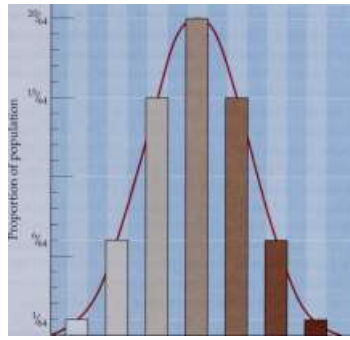
'64

o o

oo oo oo »o I«o1

ooo too • •o ••o •••

20/



Skin pigmentation

10.17 Polygenes Determine Human Skin Pigmentation

A model of polygenic inheritance based on three genes. Alleles A, B, and C contribute melanin to the skin, but alleles a, b, and c do not. The more A, B, and C alleles an individual possesses, the darker that person's skin will be. If both members of a couple have intermediate pigmentation (in this example, AaBbCc), it is unlikely (but not impossible) that their children will have either very light or very dark skin. The actual number of genes involved is much higher.

temperatures, the new fur that grows in is light. Conversely, removal of light fur from the back, followed by local chilling of the area, causes the spot to fill in with dark fur.

It is sometimes possible to determine the proportion of individuals in a group with a given genotype that actually show the expected phenotype. This proportion is called the *penetrance* of the genotype. The environment may also affect the expressivity of the genotype—that is, the degree to which it is expressed in an individual. For an example of environmental effects on expressivity, consider how Siamese cats kept indoors or outdoors in different climates might look.

Uncertainty over how much of the phenotypic variation we observe is due to the environment and how much to the effects of polygenes complicates the analysis of quantitative inheritance. A useful approach that avoids this difficulty is to study identical twins, which develop from the same fertilized egg. Since such twins are genetically identical, any differences between them can be attributed to environmental effects.

190 CHAPTER TEN

Genes and Chromosomes

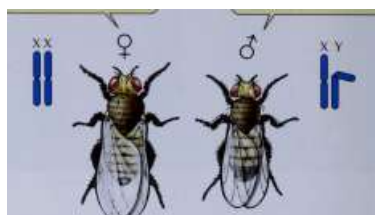
The recognition that genes occupy characteristic positions on chromosomes and thus are segregated by meiosis enabled Mendel's successors to provide a physical explanation for his model of inheritance. It soon became apparent that the association of genes with chromosomes has other genetic consequences as well.

In this section we will address the following questions: What is the pattern of inheritance of genes that occupy nearby loci on the same chromosome? How do we determine the order of genes on a chromosome—and the distances between them? Why were all the carriers of hemophilia in Queen Victoria's family women, and why were all of her descendants who had hemophilia men?

The answers to these and many other genetic questions were worked out in studies of the fruit fly *Drosophila melanogaster* (Figure 10.18). Its small size, its ease of cultivation, and its short generation time made this animal an attractive experimental system. In 1909, Thomas Hunt Morgan and his students established *Drosophila* as a highly useful laboratory organism in Columbia University's famous "fly room," where they discovered the phenomena described in this section. *Drosophila* remains extremely important in studies of chromosome structure, population genetics, the genetics of development, and the genetics of behavior.

The mirror of Venus, the Roman goddess of beauty, is the symbol for female.

The shield and spear of Mars, the Roman god of war, is the symbol for male.



10.18 *Drosophila melanogaster*, the Star of Morgan's Fly Room

The fruit fly (whose Latin name means "vinegar-loving, dark-bodied fly") has a short generation time—a major reason for its widespread use as a laboratory organism in genetics experiments.

BbVgvg

Wild type (brown

body, normal wings)

Genes on the same chromosome are linked

In the immediate aftermath of the rediscovery of Mendel's laws, the second law—independent assortment—was considered to be generally applicable. However, some investigators, including R. C. Punnett (the inventor of the Punnett square), began to observe strange deviations from the expected 9:3:3:1 ratio in some dihybrid crosses. T. H. Morgan, too, obtained data not in accord with Mendelian ratios, and specifically not in accord with the law of independent assortment.

Morgan crossed *Drosophila* of two known genotypes, BbVgvg x bbvgvg, in which B, the wild type (gray body), is dominant over b (black body), and Vg (wild-type wing) is dominant over vg (vestigial, a very small wing). (Do you recognize this type of cross? It is a test cross for the two gene pairs—see Figure 10.6.) Morgan expected to see four phenotypes in a ratio of 1:1:1:1, but this was not what he observed. The body color gene and the wing size gene were not assorting independently; rather, they were for the most part inherited together (Figure 10.19).

These results became understandable to Morgan when he assumed that the two genes are on the same chromosome —

EXPERIMENT

Question: Do alleles for different characteristics always assort independently?



X



bbvgvg (Black body, vestigial wings)

8

These are the results expected from Mendel's second law (independent assortment)...

Genotypes

Expected results

Observed genotypes



Parental

phenotypes A

Recombinant phenotypes

^

.but the actual results were inconsistent with the law.

Conclusion: These two genes do not assort independently. They are linked together on the same chromosome.

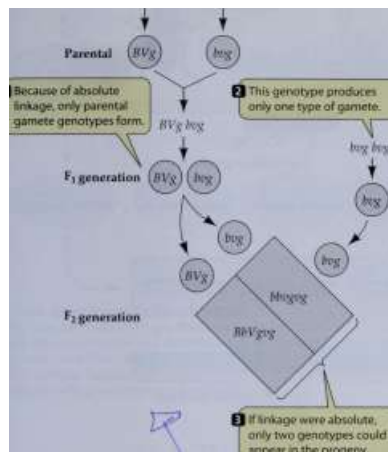
10.19 Alleles That Do Not Assort Independently

Morgan's studies showed that the genes for body color and wing size in *Drosophila* are linked, so their alleles do not assort independently. Linkage accounts for the departure of the phenotype ratios observed from the results predicted by Mendel's laws.

BBVgVg X bbvgvg

Parental

Q Because of absolute linkage, only parental gamete genotypes form.



F2 generation

If linkage were absolute, only two genotypes could appear in the progeny.

10.20 If Linkage Were Absolute

If two genes are absolutely linked on the same chromosome, all the F₂ offspring from a dihybrid test cross would have parental genotypes. If the genes in Morgan's experiment had been absolutely linked, they would have been inherited as if they were a single gene.

that is, that they are linked. After all, since the number of genes in a cell far exceeds the number of chromosomes, each chromosome must contain many genes. The full set of loci on a given chromosome constitutes a linkage group. The number of linkage groups in a species equals the number of homologous chromosome pairs.

Suppose, now, that the Bb and Vgvg loci are indeed located on the same chromosome. If we assume that the linkage is absolute, we expect to see just two types of progeny from Morgan's test cross (Figure 10.20). These two would resemble the original (grand)parents. However, this is not always the case.

Genes can be exchanged between chromatids

Absolute linkage is extremely rare. If linkage were absolute, Mendel's second law (independent assortment of alleles at different loci) would apply only to loci on different chromosomes. What actually happens is more complex, and therefore more interesting. The chromosome is not unbreakable, so recombination of genes can occur. Genes at different loci on the same chromosome do sometimes separate from one another during meiosis.

Genes may recombine when two homologous chromosomes physically exchange corresponding segments during prophase I of meiosis—that is, by crossing over (see Figure 9.16). In other words, recombination may occur at a chiasma. Sex chromosomes are paired up during meiosis (Figure 10.21).

GENETICS: MENDEL AND BEYOND 191

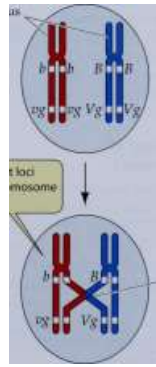
Recall that the DNA has been replicated by this stage, and that each chromosome consists of two sister chromatids. The exchange event involves only two of the four chromatids, one from each member of the chromosome pair. The chiasma can occur at any point along the length of the chromosome. The chromosome sections involved are exchanged reciprocally, so both chromatids involved in crossing over become recombinant (that is, each chromatid ends up with genes from both parents).

When crossing over takes place between two linked genes, not all progeny of a cross will have the parental types. Instead, recombinant offspring appear as well, and

Without crossing over

Paired homologous chromosomes in parent

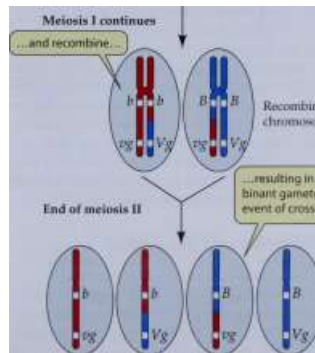
Genes at different loci on the same chromosome can separate...



Crossover (chiasma)

Meiosis I continues

Recombinant chromosomes



...resulting in two recombinant gametes from each event of crossing over.

70.2 1 Crossing Over Results in Genetic Recombination

Genes at different loci on the same chromosome can separate from one another and recombine. Crossing over occurs at a chiasma during prophase I of meiosis.

192 CHAPTER TEN

70.22 Recombinant Frequencies

The frequency of recombinant offspring (those with a phenotype different than either parent's) can be calculated. Recombinant frequencies will be larger for loci that are far apart than for those that are close together on the chromosome.

they appear in repeatable proportions called recombinant frequencies, which equal the number of recombinant progeny divided by the total number of progeny (Figure 10.22). Recombinant frequencies will be greater for loci that are far apart on the chromosome than for loci that are closer together, because a chiasma is more likely to cut between genes that are far apart than genes that are close together.

Wild type



Black vestigial

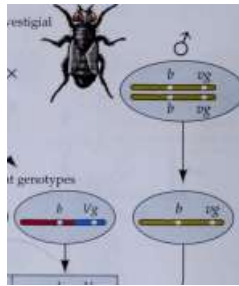
Recombination

Parental genotypes

1 X

Recombinant genotypes





^~)

Y

Parental phenotypes

Recombinant frequency =

Recombinant (nonparental) phenotypes

P

J

Geneticists make maps of eukaryotic chromosomes

If two loci are close together on a chromosome, the

odds for crossing over between them are small. In contrast,

If two loci are far apart, crossingover occurs between

them at many points. In 1911, Alfred Sturtevant, then an

undergraduate student in T. H. Morgan's fly room, realized how that simple insight could be used to show where different genes lie on the chromosome in relation to one another. He suggested that the farther apart two genes are on a chromosome, the greater the likelihood that they will separate and recombine in meiosis.

The Morgan group had determined recombinant frequencies for many pairs of linked genes. Sturtevant used these recombinant frequencies to create genetic maps that indicated the arrangement of genes along the chromosome (Figure 10.23). Ever since Sturtevant demonstrated this important point, geneticists have mapped the chromosomes of eukaryotes, prokaryotes, and viruses, assigning distances between genes in map units. A map unit corresponds to a recombinant frequency of 1%; it is also referred to as a centimorgan (cM). The founder of the fly room. You, too, can work out a genetic map (Figure 10.24).

Sex Determination and Sex-Linked Inheritance

In Kolreuter's experience, and later in Mendel's, reciprocal crosses apparently always gave identical results. The reason is that in diploid organisms, chromosomes come in pairs. One member of each chromosome pair derives from each parent; it does not matter, in general, whether a dominant allele was contributed by the mother or by the father. But sometimes the parental origin of a chromosome does matter. To understand the types of inheritance in which paren-

391 recombinants 2,300 total offspring

0.17

tal origin is important, we must consider the ways in which sex is determined in different species.

Sex is determined in different ways in different species

In corn, a plant much studied by geneticists, every diploid adult has both male and female reproductive structures. The two types of tissue are genetically identical, just as roots and leaves are genetically identical. Plants such as maize and Mendel's pea plants, and animals such as earthworms, which produce both male and female gametes in the same organism are said to be monoecious (from the Greek for "one house"). Other plants, such as date palms and oak trees, and most animals are dioecious ("two houses"), meaning that some individuals carry/produce only male gametes and the others can produce only female gametes. In other words, dioecious organisms have two sexes. In most dioecious organisms, sex is determined by differences in the chromosomes, but such determination operates in different ways in different groups of organisms. For example, the sex of a honeybee depends on whether it develops from a fertilized or unfertilized egg. A fertilized egg is diploid and gives rise to a female—either a worker or a queen, depending on the diet during larval life (again, note how the environment affects the phenotype). An unfertilized egg is haploid and gives rise to a male drone:





Diploid worker Diploid queen Haploid drone

Genetic map in map units

(cM)£

Recombinant frequencies

y is chosen as an arbitrary reference point, o.



Yellow 1/ White body / eye

1/ w

in



o o.1

1/and $z_c = 0.010$

,-* -

GENETICS: MENDEL AND BEYOND 193



Vermilion Miniature eye wing

\ /

v in))



3.1 3.4

v and m = 0.030

zv and v = 0.300 y and v = 0.322 — >A w and m - 0.327

-* ►' y and m = 0.355

70.23 Steps Toward a Genetic Map

Because the chance of a recombinant genotype occurring increases the farther apart two loci fall on a chromosome, Sturtevant was able to derive this partial map of a *Drosophila* chromosome from the Morgan group's data on the recombinant frequencies of five recessive traits. He assigned an arbitrary unit of distance—the map unit, or centimorgan (cM)—equivalent to a recombinant frequency of 0.01.

Rudimentary wing



5.8

v and r = 0.269

Q At the outset, we have no idea of the individual distances, and there are several possible sequences (a-b-c, a-c-b, b-a-c).

We make a cross AABB X aabb, and obtain an F₂ generation with a genotype AaBb. We test cross these AaBb individuals with aabb. Here are the genotypes of the first 1,000 progeny:

450 AaBb, 450 aabb, QAabk and 50 qaBb.

Q How far apart are the a and b genes? Well, what is the recombinant frequency? Which are the recombinant types, and which are the parental types?

Recombinant frequency (a to b) = $(50 + 50)/1,000 = 0.1$ So the map distance is

Map distance = $100 \times \text{recombinant frequency} = 100 \times 0.1 = 10 \text{ cM}$

o a b

l> 10 cM J

o Now we make a cross AACC X aacc, obtain an F₂ generation, and test cross it, obtaining:

460 AaCc, 460 aacc, 40 Aacc, and 40 aaCc.

How far apart are the a and c genes?

Recombinant frequency (a to c) = $(40 + 40)/1,000 = 0.08$ Map distance = $100 \times \text{recombinant frequency} = 100 \times 0.08 = 8 \text{ cM}$

(E

70.24 Map These Genes

We want to determine the order of three loci {a, b, and c} on a chromosome, as well as the map distances (in cM) between them. How do we determine a map distance?

Q How far apart are the b and c genes?

We make a cross BBCC X bbcc, obtain an F₂ generation, and test cross it, obtaining:

490 BbCc, 490 bbcc, 10 Bbcc, and 10 bbCc.

Determine the map distance between b and c.

Recombinant frequency (b to c) = $(10 + 10)/1,000 = 0.02$ Map distance = $100 \times \text{recombinant frequency} = 100 \times 0.02 = 2 \text{ cM}$

(T

h

2cM-

o Which of the three genes is between the other two?

Because a and b are the farthest apart, c must be between them.

10 cM

8cM

2cM

H

These numbers add up perfectly, but in most real cases they don't add up perfectly because of multiple crossovers.

194 CHAPTER TEN

In many other animals, including humans, sex is determined by a single sex chromosome (or by a pair of them). Both males and females have two copies of each of the rest of the chromosomes, which are called autosomes.

Female grasshoppers, for example, have two X chromosomes, whereas males have only one. Female grasshoppers are described as being XX (ignoring the autosomes) and males as XO (pronounced "ex-oh"):



'<u>

a.

N

Females form eggs that contain one copy of each autosome and one X chromosome. Males form approximately equal amounts of two types of sperm: One type contains one copy of each autosome and one X chromosome; the other type contains only autosomes. When an X-bearing sperm fertilizes an egg, the zygote is XX, and develops into a female. When a sperm without an X fertilizes an egg, the zygote is XO, and develops into a male. This chromosomal mechanism ensures that the two sexes are produced in approximately equal numbers.

As in grasshoppers, female mammals have two X chromosomes and males have one. However, male mammals also have a sex chromosome that is not found in females: the Y chromosome. Females may be represented as XX and

males as XY:

xx

II

9



Males produce two kinds of gametes: Each has a complete set of autosomes, but half the gametes carry an X chromosome and the other half carry a Y. When an X-bearing sperm fertilizes an egg, the resulting XX zygote is female; when a Y-bearing sperm fertilizes an egg, the resulting XY zygote is male.

Some subtle but important differences show up clearly in mammals with abnormal sex chromosome constitutions. These conditions, which result from nondisjunctions, as described in Chapter 9, tell us something about the functions of the X and Y chromosomes. In humans, XO individuals sometimes appear. Human XO individuals are females who are physically moderately abnormal but mentally normal; usually they are sterile. The XO condition in humans is called Turner syndrome; it is the only known case in which a human can survive with only one member of a chromosome pair (here, the X pair), although most XO conceptions terminate spontaneously early in development. XXY individuals also occur; this condition is known as Klinefelter syndrome. People with this genotype are sometimes taller than average, always sterile, and always infertile.

The X and Y chromosomes have different functions

The gene that determines maleness was identified through

observations of people with chromosomal abnormalities. For example, some XY individuals who are phenotypically women have been identified and studied; in these people, a small portion of the Y chromosome was missing. In other cases, men who were genetically XX had a small piece of the Y chromosome present, attached to another chromosome. The missing and present Y fragment in these two examples, respectively, contained the maleness-determining gene, which was named SRY (sex-determining region on the Y chromosome).

The SRY gene codes for a protein involved in primary sex determination—that is, the determination of the kinds of gametes that will be produced and the organs that will make them. In the presence of functional SRY protein, the embryo develops sperm-producing testes. If SRY protein is absent, the primary sex determination is female: ovaries and eggs develop. In this case, a gene on the X chromosome called DAX1 produces an anti-testis factor. So the role of SRY in a male is to inhibit the maleness inhibitor

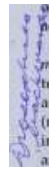
made by DAX1. The Y chromosome is the sex-determining region on the Y chromosome.

Primary sex determination is not the same as secondary

sex determination, which results in the outward manifestations of maleness and femaleness (body type, breast development, body hair, and voice). These outward characteristics are not determined directly by the presence or absence of the Y chromosome. Rather, they are determined by the actions of hormones, such as testosterone and estrogen."

The Y chromosome functions differently in *Drosophila melanogaster*. Superficially, *Drosophila* follows the same pattern of sex determination as mammals—females are XX and males are XY. However, XO individuals are males (rather than females as in mammals) and almost always are indistinguishable from normal XY males except that they are sterile. XXY individuals are normal, fertile females:

■ V*



XX



Fertile

Sterile Fertile

Fertile

Thus, in *Drosophila*, sex is determined strictly by the ratio of X chromosomes to autosome sets. If there is one X chromosome for each set of autosomes, the individual is a female; if there is only one X chromosome for the two sets of autosomes, the individual is male. The Y chromosome plays no sex-determining role in *Drosophila*, but it is needed for male fertility.

In birds, moths, and butterflies, sex is determined by the ratio of X chromosomes to autosomes. In these organisms, the female has two X chromosomes (XX) and the male has one X and one Y chromosome (XY). To avoid confusion, these forms are usually expressed as ZZ (female) and ZW (male).

expressed as ZZ

ZW



In these organisms, the female produces two types of gametes. Thus the egg determines the sex of the offspring, rather than the sperm, as in humans and fruit flies.

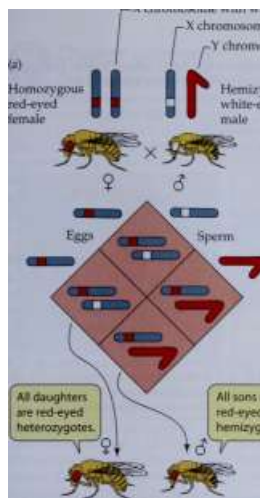
X chromosome with wild-type allele

X chromosome with allele for white eyes ~ Y chromosome (no allele at all)

Hemizygous

white-eyed

male



(b)

Homozygous

white-eyed

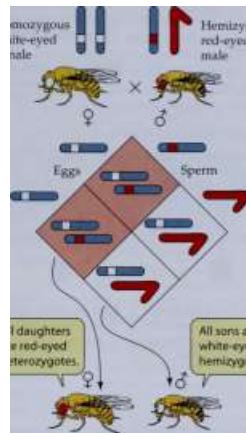
female

All sons are

red-eyed

hemizygotes.

6^



GENETICS: MENDEL AND BEYOND 195

Hemizygous

red-eyed

male

70.25 An Eye Color Is a Sex-Linked Trait in Drosophila

Thomas Hunt Morgan demonstrated that a mutant allele that causes white eyes in *Drosophila* is carried on the X chromosome.

All daughters are red-eyed heterozygotes.

All sons are white-eyed hemizygotes.

Genes on sex chromosomes are inherited in special ways

In *Drosophila* and in humans, the Y chromosome carries few known genes, but a substantial number of genes affecting a great variety of characters are carried on the X chromosome. The result of this arrangement is a deviation from the usual Mendelian ratios for the inheritance of genes located on the X chromosome. Any such gene is present in two copies in females, but in only one copy in males. Therefore, females may be heterozygous for genes that are on the X chromosome, but males will always be hemizygous for genes on the X chromosome—they will have only one of each and it will be expressed.

Kolreuter's historic reciprocal crosses, mentioned at the beginning of this chapter, always gave the same outcome regardless of which parent displayed which trait. However, reciprocal crosses do not give identical results for characters whose genes are carried on the sex chromosomes. This is a sharp deviation from the rules governing the inheritance of alleles on autosomes.

The first and still one of the best examples of sex-linked inheritance—inheritance of characters governed by loci on the sex chromosomes—is that of eye color in *Drosophila*. The wild-type eye color of these flies is red. In 1910, Morgan discovered a mutation that causes white eyes. He experimented by crossing flies of the wild-type and mutant phenotypes. His results demonstrated that the eye color locus is on the X chromosome.

When homozygous red-eyed females were crossed with (hemizygous) white-eyed males, all the sons and daughters had red eyes, because red is dominant over white, and all the progeny had inherited a wild-type X chromosome from

their mothers (Figure 10.25a). However, in the reciprocal cross, in which a white-eyed female was mated with a red-eyed male, all the sons were white-eyed and all the daughters red-eyed (Figure 10.25b).

The sons from the reciprocal cross inherited their only X chromosome from their white-eyed mother; the Y chromosome they inherited from their father does not carry the eye color locus. The daughters, on the other hand, got an X chromosome with the white allele from their mother and an X chromosome bearing the red allele from their father; they were therefore red-eyed heterozygotes.

When Morgan mated heterozygous females with red-eyed males, he observed that half their sons had white eyes and half had red eyes, but all their daughters had red eyes. This case, eye color, was carried on the X chromosome and not on the Y.

Human beings display many sex-linked characters

The human X chromosome carries thousands of genes. The alleles at these loci follow the same pattern of inheritance as those for white eyes in *Drosophila*. One human X chromosome gene, for example, has a mutant recessive allele that leads to red-green color blindness, a hereditary disorder. Red-green color blindness appears in individuals who are homozygous or hemizygous for the mutant allele.

Pedigree analysis of X-linked recessive phenotypes (Figure 10.26) reveals the following patterns:

- The phenotype appears much more often in males than in females, because only one copy of the rare allele is needed for its expression in males, while two copies must be present in females.
- A male with the mutation can pass it on only to his daughters; all his sons get his Y chromosome.

196 CHAPTER TEN

®

Female who carries gene for phenotype of interest on one X chromosome

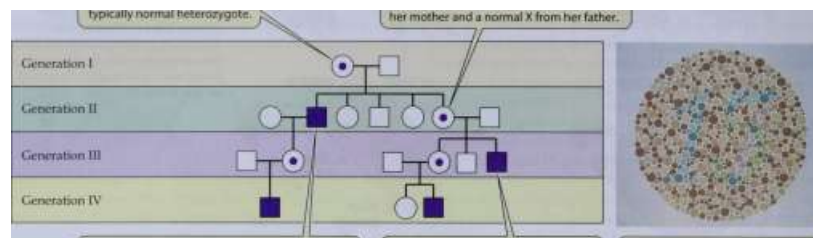
10.26 Red-Green Color Blindness is a Sex-Linked Trait in Humans

The mutant allele for red-green color blindness is inherited as an X-linked recessive.

This woman carries the mutant allele but she is a phenotypically normal heterozygote.

t

This woman inherited the mutant X from her mother and a normal X from her father.



This man inherited the mutant X from his mother and a normal Y from his father, and expresses the mutation. He passed his mutant X chromosome to his daughter, and from her to his grandson.

These siblings inherited the mutant X from their mother. The son expresses the mutation; his sister is a carrier.

In this test for red-green color blindness, people with normal color vision will see the number 15.

Daughters who receive one mutant X chromosome are heterozygous carriers. They are phenotypically normal, but they can pass the mutant X to both sons and daughters (only half of the time; half of their X chromosomes carry the normal allele). The mutant phenotype can skip a generation if the mutation passes from a male to his daughter (phenotypically normal) to her son.

As we will see in later chapters, there are several important human diseases that are inherited as X-linked recessives,

including the most common forms of muscular dystrophy and hemophilia. England's Queen Victoria was a heterozygous carrier of hemophilia A, the bleeding disorder mentioned at the beginning of this chapter. She passed it on to some of her male offspring and thereby to several of the royal families of Europe.

Human mutations inherited as X-linked dominants are rarer than recessives, because dominants appear in every generation, and because people carrying the harmful mutations, even as heterozygotes, often fail to survive and/or reproduce. (Look at the four points above and try to determine what would happen if the mutation were dominant.)

The small human Y chromosome carries only about 20 known genes. Among them are the male sex determinants, whose existence was suggested by the phenotypes of the XO and XXY individuals described on page 20. Y-linked alleles are passed from father to son. (You can verify this with a Punnett square.)

Non-Nuclear Inheritance

The nucleus is not the only organelle in a eukaryotic cell that carries genetic material. As we described in Chapter 4, mitochondria and plastids, which may have arisen from

bacteria that colonized other cells, contain small numbers of genes. For example, in humans, there are about 60,000 genes in the nucleus and 37 in mitochondria. Plastid genomes are about five times larger than those of mitochondria. In any case, the organelle genes include several that are important for organelle assembly and function, so it is not surprising that mutations of these genes have profound effects on the organism.

The inheritance of organelle genes differs from that of nuclear genes for several reasons. First, mitochondria and plastids are apparently passed on from the mother only. As you will see in later chapters, eggs contain abundant cytoplasm

and organelles, but the only part of the sperm that survives to take part in the union of haploid gametes is the nucleus. So you have inherited your mother's mitochondria (with their genes), but not your father's. Second, there may be hundreds of mitochondria and/or plastids in a cell. So a cell is not diploid for organelle genes; rather it's truly polyploid. A third factor is that organelle genes tend to mutate at much faster rates than nuclear genes, so there are multiple alleles for organelle genes.

The phenotypes of mutations in the DNA of organelles reflect the organelles' roles. For example, some mutations affect proteins that assemble chlorophyll molecules into the photosystem reaction centers (see Figure 8.11), and result in a phenotype that is essentially a white instead of a green tissue. Mitochondrial mutations that affect one of the complexes in the electron transport chain result in less ATP production. They have especially noticeable effects in tissues with a high energy requirement, such as the nervous system, muscles, and kidneys. In 1995, Greg Lemond, a professional cyclist who had won the famous Tour de France three times, was forced to retire because of muscle weakness suspected to be caused by a mitochondrial mutation.

GENETICS: MENDEL AND BEYOND 197



Chapter Summary

The Foundations of Genetics

► Plant breeders can control which plants mate. Although it has long been known that both parent plants contribute equally to the character traits of their offspring, before Mendel's time it was believed that, once they were brought together, the units of inheritance blended and could never be separated. Review Figure 10.1

► Although Gregor Mendel's work was meticulous and well documented, his discoveries, reported in the 1860s, lay dormant until decades later, when others rediscovered them.

Mendel's Experiments and Laws of Inheritance

► Mendel used garden pea plants for his studies because they were easily cultivated and crossed, and because they showed numerous characters (such as seed shape) with clearly different traits (spherical or wrinkled). Review Table 10.1

► In a monohybrid cross, the offspring showed only one of the two traits. Mendel proposed that the trait observed in the first generation (F₁) was dominant and the other was recessive. Review Table 10.1

► When the F₁ offspring were self-pollinated, the resulting F₂ generation showed a 3:1 phenotypic ratio, with the recessive phenotype present in one-fourth of the offspring. This reappearance of the recessive phenotype refuted the blending hypothesis. Review Figure 10.3

► Because some alleles are dominant and some are recessive, the same phenotype can result from different genotypes. Homozygous genotypes have two copies of the same allele; heterozygous genotypes have two different alleles. Heterozygous genotypes yield phenotypes that show the dominant trait.

► On the basis of many crosses using different characters, Mendel proposed his first law: that the units of inheritance (now known as genes) are particulate, that there are two copies (alleles) of each gene in every parent, and that during gamete formation the two alleles for a character segregate from each other. Review Figure 10.4

► Geneticists who followed Mendel showed that genes are carried on chromosomes and that alleles are segregated during meiosis I. Review Figure 10.5

► Using a test cross, Mendel was able to determine whether a plant showing the dominant phenotype was homozygous or heterozygous. The appearance of the recessive phenotype in half of the offspring of such a cross indicates that the parent is heterozygous. Review Figure 10.6

► From studies of the simultaneous inheritance of two characters, Mendel concluded that alleles of different genes assort independently. Review Figures 10.7, 10.8

► We can predict the results of hybrid crosses either by using a Punnett square or by calculating probabilities. To determine the joint probability of independent events, we multiply the individual probabilities. To determine the probability of an event that can occur in two or more different ways, we add the individual probabilities. Review Figure 10.9

► That humans exhibit Mendelian inheritance can be inferred by the analysis of pedigrees. Review Figures 10.10, 10.11

Alleles and Their Interactions

► New alleles arise by mutation, and many genes have multiple alleles. Review Figure 10.12

► Dominance is usually not complete, since both alleles in a heterozygous organism may be expressed in the phenotype. Review Figures 10.13, 10.14

Gene Interactions

- In epistasis, the products of different genes interact to produce a phenotype. Review Figure 10.15
- In some cases, the phenotype is the result of the additive effects of several genes (polygenes), and inheritance is quantitative. Review Figure 10.17
- Environmental variables such as temperature, nutrition, and light affect gene action.

Genes and Chromosomes

- Each chromosome carries many genes. Genes located on the same chromosome are said to be linked, and they are often inherited together. Review Figures 10.19,10.20
- Linked genes recombine by crossing over in prophase I of meiosis. The result is recombinant gametes, which have new combinations of linked genes because of the exchange. Review Figures 10.21,10.22
- The distance between genes on a chromosome is proportional to the frequency of crossing over between them. Genetic maps are based on recombinant frequencies. Review Figures 10.23,10.24

Sex Determination and Sex-Linked Inheritance

- Sex chromosomes carry genes that determine whether male or female gametes are produced. The specific functions of X and Y chromosomes differ among species.
- In fruit flies and mammals, the X chromosome carries many genes, but its homolog, the Y chromosome, has only a few. Males have only one allele for most X-linked genes, so rare alleles show up phenotypically more often in males than in females. Review Figures 10.25,10.26

Non-Nuclear Inheritance

- Cytoplasmic organelles such as plastids and mitochondria contain some heritable genes.
- Organelle genes are generally inherited by way of the egg (maternal inheritance), because male gametes contribute only their nucleus to the zygote at fertilization.

Some Genetics Problems

1. Using the Punnett squares below, show that for typical dominant and recessive autosomal traits, it does not matter which parent contributes the dominant allele and which the recessive allele. Cross true-breeding tall plants (TT) with true-breeding dwarf plants (tt).

Tall

Dwarf

female male

Tall male

Dwarf female

Female gametes



Male gametes

Female gametes

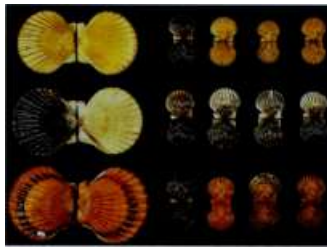


Male gametes

2. The accompanying photograph shows the shells of 15 bay scallops, *Argopecten irradians*. These scallops are hermaphroditic; that is, a single individual can reproduce sexually by self-fertilization, as did the pea plants of the F₁ generation in Mendel's experiments. Three color schemes are evident: yellow, orange, and black and white. The color-determining gene has three alleles. The top row shows a yellow scallop and a representative sample of its offspring, the middle row shows a black-and-white scallop and its offspring, and the bottom row shows an orange scallop and its offspring. Assign a suitable symbol to each of the three alleles participating in color determination;

198 CHAPTER TEN

then determine the genotype of each of the three parent individuals and explain what you can about the genotypes of the different offspring. Explain your results carefully



Show diagrammatically what occurs when the F₁ offspring of the cross in Question 1 self-pollinate.

Female gametes



Male gametes

4. A new student of genetics suspects that a particular recessive trait in fruit flies (dumpy wings, which are somewhat smaller and more bell-shaped than the wild type) is sex-linked. A single mating between a fly having dumpy wings (dp; female) and a fly with wild-type wings (Dp; male) produces three dumpy-winged females and two wild-type males. On the basis of these data, is the trait sex-linked or autosomal? What were the genotypes of the parents? Explain how these conclusions can be reached on the basis of so few offspring.

5. The sex of fishes is determined by the same XY system as in humans. An allele at one locus on the Y chromosome of the fish *Lebistes* causes a pigmented spot to appear on the dorsal fin. A male fish that has a spotted dorsal fin is mated with a female fish that has an unspotted fin. Describe the phenotypes of the F₁ and the F₂ generations from this cross.

6. In *Drosophila melanogaster*, the recessive allele p, when homozygous, determines pink eyes. Pp or PP results in wild-type eye color. Another gene, on another chromosome, has a recessive allele, sw, that produces short wings when homozygous. Consider a cross between females of genotype PPSwSw and males of genotype ppsiusw. Describe the phenotypes and genotypes of the F₁ generation and of the F₂ generation produced by allowing the F₁ progeny to mate with one another.

7. On the same chromosome of *Drosophila melanogaster* that carries the p (pink eyes) locus, there is another locus that affects the wings. Homozygous recessives, byby, have blis-tery wings, while the dominant allele By produces wild-type wings. The P and By loci are very close together on the chromosome; that is, they are tightly linked. In answering the following questions, assume that no crossing over occurs.

- For the cross PPByBy x ppbyby, give the phenotypes and genotypes of the F₁ generation and of the F₂ generation produced by interbreeding of the F₁ progeny.
- For the cross PPbyby x ppByBy, give the phenotypes and genotypes of the F₁ and F₂ generations.
- For the cross in Question 7b, what further phenotype(s) would appear in the F₂ generation if crossing over occurred?
- Draw a nucleus undergoing meiosis, at the stage in which the crossing over in Question 7c occurred. In which generation (P, F₁ or F₂) did this crossing over take place?

8. Consider the following cross of *Drosophila melanogaster* with alleles as described in Question 6. Males with genotype Ppswsw are crossed with females of genotype ppSswsw. Describe the phenotypes and genotypes of the F₁ generation.

9. In the Andalusian fowl, a single pair of alleles controls the color of the feathers. Three colors are observed: blue, black, and splashed white. Crosses among these three types yield the following results:

PARENTS

PROGENY

Black x blue Black x splashed white Blue x splashed white Black x black Splashed white x splashed white

Blue and black (1:1)

Blue

Blue and splashed white (1:1)

Black

Splashed white

- What progeny would result from the cross blue x blue?
- If you wanted to sell fowl, all of which would yield blue fowl, how should you proceed?

10. In *Drosophila melanogaster*, white (w), eosin (w^e), and wild-type red (w⁺) are multiple alleles of a single locus for eye color. This locus is on the X chromosome. A female that has eosin (pale orange) eyes is crossed with a male that has wild-type eyes. All the female progeny are red-eyed; half the male offspring have eosin eyes, and half have white eyes.

a. What is the order of dominance of these alleles?

b. What are the genotypes of the parents and progeny?

11. Color blindness is a recessive trait. Two people with normal color vision have two sons, one color-blind and one with normal color vision. If the couple also has daughters, what proportion of them will have normal color vision? Explain.

12. A mouse with an agouti coat is mated with an albino mouse of genotype aabb. Half of the offspring are albino, one-fourth are black, and one-fourth are agouti. What are the genotypes of the agouti parents and of the various kinds of offspring? {Hint: See the section on epistasis.}

13. The disease Leber's optic neuropathy is caused by a mutation in a gene carried on mitochondrial DNA. What would be the result in their first child if a man with this disease married a woman who did not have the disease? What would be the result if the wife had the disease and the husband did not?



DNA and Its Role in Heredity

The image of the DNA double helix is one of the great secular icons to emerge in the last half of the twentieth century. Its elegance and simplicity make it instantly recognizable by the general public. The story of how scientists determined that the gene envisioned by Mendel is made of DNA is one of the epics of experimental biology. These studies opened up an entirely new field of natural science: molecular biology, which is concerned with DNA and its expression in cells.

The representation of DNA shown below is the familiar double helix, but with an added chemical shown in green. The green molecule is benzpyrene, one of the toxic chemicals emitted in tobacco smoke. This extra chemical entity has dire consequences. The regular, twisted structure of DNA is just the right shape to allow benzpyrene to wedge into the groove of the helix. A covalent bond forms between the benzpyrene and a DNA monomer, causing a major problem when DNA is expressed and replicated. Ultimately, there are irreversible changes in the DNA, and these changes are passed on to the two daughter cells after a cell division cycle. This damage to the DNA is the key event that begins cancer—in the case of benzpyrene, usually lung cancer.

In this and the next several chapters, we focus on the structure, replication, and function of DNA. As you will see, the structure of DNA determines its functions. This chapter first describes the key experiments that led to the determination that the genetic material is DNA. Then the structure and replication of the molecule are described. Finally, we present two practical applications that have arisen from our knowledge of DNA replication: DNA sequencing, and the polymerase chain reaction.

DNA: The Genetic Material

During the first half of the twentieth century, the hereditary material was generally assumed to be a protein. The impressive chemical diversity of proteins made this assumption seem reasonable. In addition, some proteins—notably enzymes and antibodies—showed great specificity. Nucleic

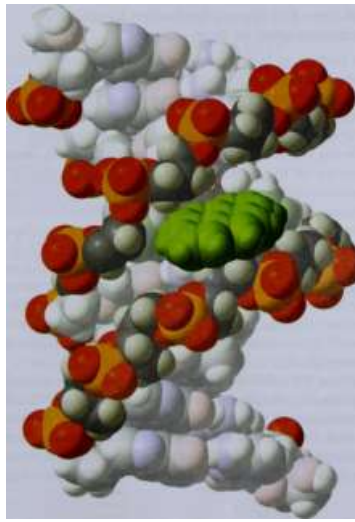
The Double Helix of DNA

A computer-generated model of DNA. The molecule in green is benzpyrene, a major cancer-causing component of tobacco smoke. The "backbone" of the DNA molecule is visible as a chain of sugars (gray) and phosphate groups (red and orange).

acids, by contrast, were known to have only a few components and seemed too simple to carry the complex information expected in the genetic material.

Circumstantial evidence, however, pointed to DNA. It was in the right place, since it was an important component of the nucleus and chromosomes, which were known to carry genes. And it was present in the right amounts. During the 1920s, a dye was developed that bound specifically to DNA and turned red in direct proportion to the amount of DNA present. When different cells were stained with this dye and their color intensity measured, each species appeared to have its own specific nuclear DNA content. Furthermore, the quantity in somatic cells was twice that in eggs or sperm—as might be expected for diploid and haploid cells, respectively. These two observations were consistent with DNA as the genetic material.

But circumstantial evidence is not a scientific demonstration of cause and effect. After all, proteins are also present in nuclei. The convincing demonstration that DNA is the genetic material came from two lines of experiments, one on bacteria and the other on bacterial viruses.



200 CHAPTER ELEVEN

EXPERIMENT

Question: Can an extract from dead bacterial cells genetically transform living bacterial cells?

The virulent S strain bacteria are killed by heating.

METHOD

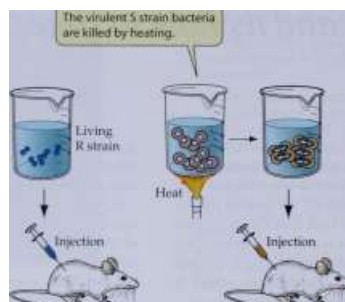
S strain

RESULTS



Mouse dies

Living S strain cells isolated from heart



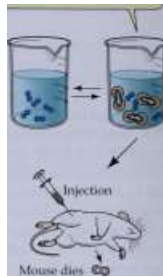
Dead S strain cells are mixed with living, nonvirulent R strain bacteria.

Mouse healthy

No bacterial cells found in heart

Mouse healthy

No bacterial cells found in heart



Mouse dies

Living S strain cells isolated from heart

Conclusion: A chemical component from one cell is capable of genetically transforming another cell.

7.7.7 Genetic Transformation of Nonvirulent R Pneumococci Frederick Griffith's experiments demonstrated that something in the virulent S strain could transform nonvirulent R strain bacteria into a lethal form, even when the S strain bacteria had been killed by high temperatures.

DNA from one type of bacterium genetically transforms another type

The history of biology is filled with incidents in which research on one specific topic has—with or without answering the question originally asked—contributed richly to another, apparently unrelated area. Such a case of "serendipity" is the work of Frederick Griffith, an English physician.

In the 1920s, Griffith was studying the disease-causing behavior of the bacterium *Streptococcus pneumoniae*, or pneumococcus, one of the agents that causes pneumonia in humans. He was trying to develop a vaccine against this devastating illness (antibiotics had not yet been discovered). He was working with two strains of pneumococcus. A bacterial strain is a population of cells descended from a single parent cell; strains differ in one or more inherited characteristics. Griffith's strains were designated S and R because, when grown in the laboratory, one produces shiny, smooth (S) colonies, and the other produces colonies that look rough (R).

When the S strain was injected into mice, the mice died within a day, and the hearts of the dead mice were found to be teeming with the deadly bacteria. When the R strain was injected, the mice did not become diseased. In other words, the S strain is virulent (disease-causing) and the R strain is nonvirulent. The virulence of the S strain is caused by a

polysaccharide capsule that protects the bacterium from the immune defense mechanisms of the host. The R strain lacks this capsule, so the R strain cells can be inactivated by the defenses of a mouse.

With the hope of developing a vaccine against pneumonia, Griffith inoculated some mice with heat-killed S pneumococci. These heat-killed bacteria did not produce infection. However, when Griffith inoculated other mice with a mixture of living R bacteria and heat-killed S bacteria, to his astonishment, the mice died of pneumonia. When he examined blood from the hearts of these mice, he found it full of living bacteria—many of them with characteristics of the virulent S strain! Griffith concluded that, in the presence of the dead S pneumococci, some of the living R pneumococci had been transformed into virulent S-strain organisms (Figure 11.1).

We now call the phenomenon of the genetic alteration of an organism transformation. In terms of Griffith's observations, one could say that transformation is the uptake of information from the environment. As we'll see, today's definition of transformation is more precise. For now, note that living R pneumococci had gained a trait—virulence—from something in their environment.

Did this transformation of the bacteria depend on something the mouse did? No. It was shown that simply incubating living R and heat-killed S bacteria together in a test tube yielded the same transformation. Next it was discovered that a cell-free extract of heat-killed S cells also transformed R cells. (A cell-free extract contains all the contents of ruptured cells, but no intact cells.) This result demonstrated that some substance—called at the time a chemical transforming principle—from the dead S pneumococci

could cause a heritable change in the affected R cells. From these observations, some scientists concluded that this transforming principle carried heritable information, and thus was the genetic material.

The transforming principle is DNA

The identification of the transforming principle was a crucial step in the history of biology, accomplished over a period of several years by Oswald T. Avery and his colleagues at what is now Rockefeller University. They treated samples of the transforming principle in a variety of ways to destroy different types of substances—proteins, nucleic acids, carbohydrates, and lipids—and tested the treated samples to see if they had retained transforming activity.

The answer was always the same: If the DNA in the sample was destroyed, transforming activity was lost; everything else was dispensable. As a final step, Avery, with Colin MacLeod and Maclyn McCarty, isolated virtually pure DNA from a sample of pneumococcal transforming principle and showed that it caused bacterial transformation.

The work of Avery, MacLeod, and McCarty, published in 1944, was a milestone in establishing that DNA is the genetic material in cells. However, it had little impact at the time, for two reasons. First, most scientists did not believe that DNA was

chemically complex enough to be the hereditary material, especially given the great chemical complexity of proteins. Second, and perhaps more important, it was not yet obvious that bacteria even had genes; bacterial genetics was still to be elucidated (see Chapter 13).

Viral replication experiments confirm that DNA is the genetic material

A report published in 1952 by Alfred D. Hershey and Martha Chase of the Carnegie Laboratory of Genetics had a much greater immediate impact than did Avery's 1944 paper. The Hershey-Chase experiment was carried out with a virus that infects bacteria. This virus, called T2 bacteriophage, consists of little more than a DNA core packed inside a protein coat (Figure 11.2a). The virus is thus made of the two materials that were, at the time, the leading candidates for the genetic material.

When a T2 bacteriophage attacks a bacterium, part (but not all) of the virus enters the bacterial cell. Hershey and Chase set out to determine which part of the virus—protein or DNA—enters the bacterial cell. To trace these two components during the life cycle of the virus (Figure 11.2b), Hershey and Chase labeled each with a specific radioactive tracer.

All proteins contain some sulfur (in the amino acids cysteine and methionine), an element not present in DNA, and sulfur has a radioactive isotope, ^{35}S . The deoxyribose-phosphate "backbone" of DNA, on the other hand, is rich in phosphorus (see Chapter 3), an element that is not present in most proteins—and phosphorus also has a radioactive isotope, ^{32}P . Hershey and Chase grew one batch of T2 in a bacterial culture in the presence of ^{32}P , so that all the viral DNA was labeled with ^{32}P . Similarly, all the proteins of another batch of T2 were labeled with ^{35}S .

(a) The virus: T2 bacteriophage

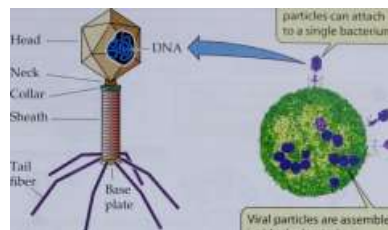
Head

Neck

Collar

Sheath

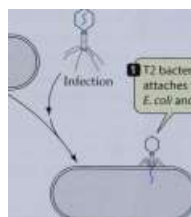
Many bacteriophage particles can attach to a single bacterium.



Viral particles are assembled inside the bacterium.

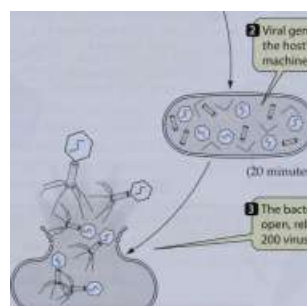
(b) Life cycle of the T2 bacteriophage

E. coli 15



T2 bacteriophage attaches to the surface of *E. coli* and injects its DNA.

o Viral genes take over the host's synthetic machinery.



The bacterium breaks open, releasing about 200 viruses.

7.2.12 and the Bacteriophage Reproduction Cycle

(a) The external structures of the bacteriophage T2 consist entirely of protein. This cutaway view shows a strand of DNA within the head. (b) T2 is parasitic on E. coli, depending on the bacterium to produce new viruses.

In separate experiments, Hershey and Chase combined radioactive viruses containing either ^{32}P or ^{35}S with bacteria. After a few minutes, they agitated the mixtures vigorously in a kitchen blender, which (without bursting the bacteria) stripped away the parts of the virus that had not penetrated the bacteria. Then, using a centrifuge, Hershey and Chase separated the bacteria from the rest of the mixture.

202 CHAPTER ELEVEN

EXPERIMENT

Question: Which component of a bacteriophage—DNA or protein—is the hereditary material that enters a bacterial cell to direct the assembly of new virus particles?

Experiment 1

Experiment 2

(2) T2 phages are grown in a medium containing ^{32}P (P is an element in DNA but not in proteins).

(3) T2 phages are grown in a medium containing ^{35}S (S is an element in proteins but not in DNA).

: P-containing DNA



METHOD ^{35}S -containing phage coats

The labeled phages are used to infect bacteria.

After a short time, mixing in a blender detaches viruses from cells.

Centrifuging forces the bacterial cells to the bottom of the tube, forming a pellet. Supernatant fluid with viruses is drained off.

RESULTS

The ^{32}P is in the pellet with the bacteria.

Pellet



Most of the ^{35}S is in the supernatant fluid with the viruses.

Conclusion: DNA, not protein, enters bacterial cells and directs the assembly of new virus particles.

They found that most of the ^{35}S (and thus the protein) had separated from the bacteria, and that most of the ^{32}P (the DNA) had stayed with the bacteria. These results suggested that the DNA was transferred to the bacteria, whereas the protein remained outside (Figure 11.3).

Hershey and Chase then performed similar but longer experiments, allowing a progeny generation of viruses to be collected. The resulting T2 progeny (the "offspring" of the original viruses) contained almost none of the original

Pellet

11.3 The Hershey-Chase Experiment

Because only DNA entered the bacterial cell during infection, the experiment demonstrated that DNA, not protein, is the hereditary material.

^{35}S , but about one-third of the original ^{32}P —and thus, presumably, one-third of the original DNA. Because DNA was carried over in the virus from generation to generation but protein was not, a logical conclusion was that the hereditary information of the virus is contained in the DNA.

The Hershey-Chase experiment convinced most scientists that DNA is the carrier of hereditary information. By this time, other researchers had identified mutations—and therefore genes—in viruses and bacteria.

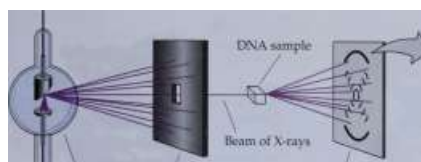
The Structure of DNA

Once scientists agreed that the genetic material is DNA, they wanted to learn its precise chemical structure. In the structure of DNA, they hoped to find the answers to two questions: how DNA is replicated between nuclear divisions, and how it causes the synthesis of specific proteins. Both expectations were fulfilled. X-ray crystallography studies provided the first clues about the dimensions of DNA and hinted that it had a helical form. Dimensionally accurate models built by James Watson and Francis Crick completed the picture.

X-ray crystallography provided clues to DNA structure

The structure of DNA was deciphered only after many types of experimental evidence and theoretical considerations were combined. The most crucial evidence was obtained by X-ray crystallography (Figure 11.4). The positions of atoms in a crystalline substance can be inferred from the pattern of diffraction of X-rays passed through it, but even today this is not an easy task when the substance is of enormous molecular weight.

In the early 1950s, even a highly talented X-ray crystallographer could (and did) look at the best available images from DNA preparations and fail to see what they meant. Nonetheless, the attempt to characterize DNA would have been impossible without the crystallographs prepared by



X-ray source Lead screen

Photographic plate

7.4 X-Ray Crystallography Revealed the Basic Helical Nature of DNA Structure

The positions of atoms in DNA can be inferred by the pattern of diffraction of X-rays passed through it, although the task requires tremendous skill.

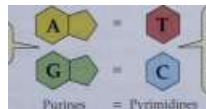
the English chemist Rosalind Franklin. Franklin's work, in turn, depended on the success of the English biophysicist Maurice Wilkins, who prepared very uniformly oriented DNA fibers, which provided samples for diffraction that were far better than previous ones.

The chemical composition of DNA was known

The chemical composition of DNA also provided important clues about its structure. Biochemists knew that DNA was a polymer of nucleotides. Each nucleotide of DNA consists of a molecule of the sugar deoxyribose, a phosphate group, and a nitrogen-containing base (see Figures 3.16 and 3.17). The only differences among the four nucleotides of DNA are their nitrogenous bases: the purines adenine (A) and guanine (G), and the pyrimidines cytosine (C) and thymine (T).

In 1950, Erwin Chargaff at Columbia University reported some observations of major importance. He and his colleagues found that DNA from many different species—and from different sources within a single organism—exhibits certain regularities. In almost all DNA the following rule holds: The amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine (Figure 11.5). As a result, the total abundance of purines equals the total abundance of pyrimidines. The structure of DNA could not have been worked out without this information, yet its significance was overlooked for at least three years. Interestingly, the ratio of A + T to G + C

In DNA, the amount of purines (A + G)...



...is always equal to the amount of pyrimidines (T + C).

Purines



These spots are caused by diffracted X-rays.

varies widely among different organisms (Table 11.1). This observation reinforced the importance of Chargaff's rule.

Watson and Crick described the double helix

The solution to the puzzle of the structure of DNA was accelerated by the technique of model building: assembling three-dimensional representations of possible molecular structures using known relative molecular dimensions and known bond angles. This technique, originally exploited in structural studies by the American chemist Linus Pauling, was used by the English physicist Francis Crick and the American geneticist James D. Watson, then both at the Cavendish Laboratory of Cambridge University.

Watson and Crick attempted to combine all that had been learned so far about DNA structure into a single coherent model. The crystallographers' results (see Figure 11.4) convinced Watson and Crick that the DNA molecule is helical (cylindrically spiral) and provided the values of certain distances within the helix. The results of density measurements and previous model building suggested that there are two polynucleotide chains in the molecule. The modeling studies had also led to the conclusion that the two chains in DNA run in opposite directions—that is, that they are antiparallel. (We'll clarify this point in the next section.)

Crick and Watson built several models. Late in February of 1953, they built the one that established the general structure of DNA. There have been minor amendments to their first published structure, but the principal features remain unchanged.

1U

Percentages of Bases in the DNA of Some Well-Studied Species

DNA ORIGIN

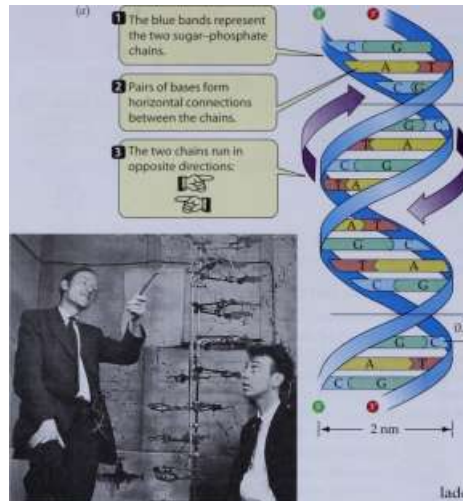
AMOUNT OF BASE (PERCENTAGE OF TOTAL DNA)

go ® €D ®

7.5 Chargaff's Rule

The total abundances of purines and pyrimidines are equal in DNA.

204 CHAPTER ELEVEN



(b)

Phosphorus

Carbon in

sugar-phosphate

"backbone"

Hydrogen Oxygen

3.4 nm

0.34 nm

Bases- =

7.6 DNA Is a Double Helix

(a) Watson and Crick proposed that DNA is a double helical molecule, (b) Biochemists can now pinpoint the position of every atom in a DNA macromolecule. To see that the essential features of the original Watson-Crick model have been verified, follow with your eyes the double helical ribbons of sugar-phosphate groups and note the horizontal rungs of the bases (see also Figure 3.18).

Four key features define DNA structure

Four features summarize the molecular architecture of DNA. The DNA molecule is

- ▶ a double-stranded helix,
- ▶ of uniform diameter,
- ▶ right-handed (that is, it twists to the right, as do the threads on most screws), and
- ▶ antiparallel (the two strands run in opposite directions).

The sugar-phosphate backbones of the polynucleotide chains coil around the outside of the helix, and the nitrogenous bases point toward the center (Figure 11.6).

The two chains are held together by hydrogen bonding between specifically paired bases. Consistent with Chargaff's rule, adenine (A) pairs with thymine (T) by forming two hydrogen bonds, and guanine (G) pairs with cytosine (C) by forming three hydrogen bonds (Figure 11.7). Every base pair consists of one purine (A or G) and one pyrimidine (C or T). This pattern is known as complementary base pairing. Because the AT and GC pairs, like rungs of a



Minor groove

Major groove

ladder, are of equal length and fit identically into the double helix, the diameter of the helix is uniform. The base pairs are flat, and their stacking in the center of the molecule is stabilized by hydrophobic interactions (see Chapter 2), contributing to the overall stability of the double helix.

What does it mean to say that the two DNA strands run in opposite directions? The direction of a polynucleotide can be defined by looking at the linkages (called phospho-diester bonds) between adjacent nucleotides. In the sugar-phosphate backbone of DNA, the phosphate groups connect to the 3' carbon of one deoxyribose molecule and the 5' carbon of the next, linking successive sugars together (see Figure 11.7). The prime (') designates the position of a carbon atom in the five-carbon sugar deoxyribose.

Thus the two ends of a polynucleotide differ. Polynucleotides have a free (not connected to another nucleotide) 5' phosphate group (—OPO_3) at one end, called the 5' end; a free 3' hydroxyl group (—OH) is at the other, the 3' end. The 5' end of one strand in a DNA double helix is paired with the 3' end of the other strand, and vice versa; in other words, the strands run in opposite directions .

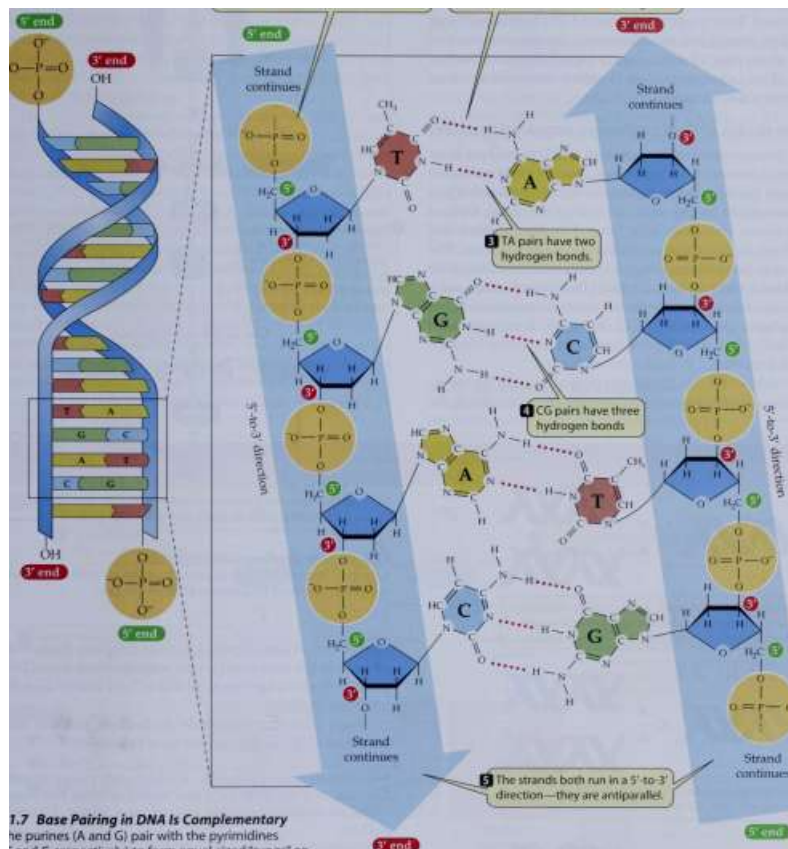
The double helical structure of DNA is essential to its function

The genetic material must perform four important functions, and the DNA molecule modeled by Watson and Crick was elegantly suited to three of them.

► The genetic material should be able to store an organism's genetic information. With its millions of nucleotides in a sequence that differs in every species and every individual, DNA fits this role nicely.

Q Each phosphate group links the 3' carbon of one sugar to the 5' carbon of the next sugar along the backbone.

o Pairs of complementary bases form hydrogen bonds that hold the two strands of the DNA double helix together.



11.7 Base Pairing in DNA Is Complementary

The purines (A and G) pair with the pyrimidines (T and C, respectively) to form equal-sized "rungs" on a "ladder" (the sugar-phosphate backbones). The ladder is twisted into a double helical structure.

The genetic material must be susceptible to mutation, or permanent changes in its information. For DNA, mutations might be simple changes in the linear sequence of nucleotide pairs.

The genetic material must be precisely replicated in the cell division cycle. Replication could be accomplished by

complementary base pairing, A with T and G with C. In the original publication of their findings in the journal *Nature* in 1953, Watson and Crick coyly pointed out, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." The genetic material must be expressed as the phenotype. This function is not inherent in the structure of DNA; however, as we show in the next chapter, it also turns out to be well served by DNA.

206 CHAPTER ELEVEN

rn



DNA Replication

Watson and Crick's model for DNA replication was soon confirmed. First, experiments showed DNA replicated from template strands in a test tube containing simple substrates and an enzyme. Then an elegant experiment showed that each of the two strands of the double helix serves as a template for a new strand.

Three modes of DNA replication appeared possible

Just three years after Watson and Crick published their paper in *Nature*, their prediction that the DNA molecule contains the information needed for its own replication was demonstrated by the work of Arthur Kornberg, then at Washington University in St. Louis. Kornberg showed that DNA can replicate in a test tube with no cells present. The only requirements are DNA, a specific enzyme (which he obtained from bacteria and called DNA polymerase), and a mixture of four precursors: the deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and dTTP. If any one of the four deoxyribonucleoside triphosphates is omitted from the reaction mixture, DNA does not replicate.

Somehow, the DNA itself serves as a template for the reaction—a guide to the exact placement of nucleotides in the

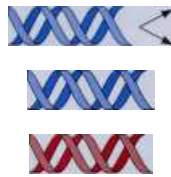
Original DNA

After one round of replication



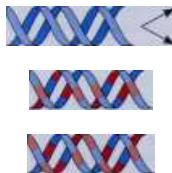
Semiconservative replication would produce molecules with both old and new DNA, but each molecule would contain one complete old strand and one new one.

(b)



Conservative replication would preserve the original molecule and generate an entirely new molecule.

(c)



Dispersive replication would produce two molecules with old and new DNA interspersed along each strand.

11.8 Three Models for DNA Replication

In each model, original DNA is shown in blue and newly synthesized DNA in red.

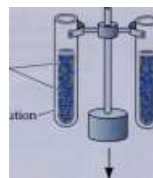
RESEARCH METHOD

Before centrifugation

DNA

CsCl

in solution



After a brief period of centrifugation



Q When a solution of cesium chloride is centrifuged at extremely high speed, the cesium ions tend to sink slightly, forming a density gradient along the tube.

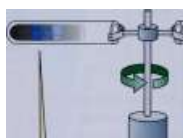


11 Labeled ("heavy") DNA separates from lighter DNA according to their different densities.

I

After equilibrium is reached

Mixture



n

Unlabeled ("light")

Labeled ("heavy")

DNA samples of different densities float at the points where their densities match that of the CsCl gradient.

11.9 Density Gradient Centrifugation

Labeled ("heavy") DNA will separate from lighter DNA in the density gradient formed by a cesium chloride solution.

new strand. Where there is a T in the template, there must be an A in the new strand, and so forth. How does DNA perform the template function; that is, how exactly does the molecule replicate?

There were three possible replication patterns that would result in complementary base pairing:

- ▶ Semiconservative replication, in which each parent strand serves as a template for a new strand and the two new DNA's each have one old and one new strand (Figure 11.8a)
- ▶ Conservative replication, in which the original double helix serves as a template for, but does not contribute to, the new double helix (Figure 11.8fr)
- ▶ Dispersive replication, in which fragments of the original DNA molecule serve as templates for assembling two molecules, each containing old and new parts, perhaps at random (Figure 11.8c)

Watson and Crick's original paper suggested that DNA replication was semiconservative, but Kornberg's experiment did not provide a basis for choosing among these three models.

DNA AND ITS ROLE IN HEREDITY 207

Meselson and Stahl demonstrated that DNA replication is semiconservative

A clever experiment conducted by Matthew Meselson and Franklin Stahl convinced the scientific community that semiconservative replication is the correct model. Working at the California Institute of Technology in 1957, they devised a simple way to distinguish old strands of DNA from new ones: density labeling.

The key to their experiment was the use of a "heavy" isotope of nitrogen. Heavy nitrogen (^{15}N) is a rare, nonradioactive isotope that makes molecules containing it more dense than chemically identical molecules containing the common isotope, ^{14}N . To distinguish DNA of different densities (that is, DNA containing ^{15}N versus DNA containing ^{14}N), Meselson, Stahl, and Jerome Vinograd invented a new centrifugation procedure using a cesium chloride (CsCl) solution.

Spinning solutions or suspensions at high speed in a centrifuge causes the solutes or particles to separate, and they form a gradient according to their density. A concentrated solution of CsCl has a density very close to that of DNA. At high gravitational forces, cesium ions sediment out of the solution to some extent, establishing a gradient from low density at the top of the centrifuge tube to high density at the bottom. When a DNA sample is dissolved in

CsCl and centrifuged at about 100,000 times the force of gravity, the DNA gathers in a band at a position in the tube where the density of the CsCl solution equals its own density (Figure 11.9).

After developing this method of distinguishing DNA densities, Meselson and Stahl grew a culture of the bacterium *Escherichia coli* for 17 generations in a medium in which the nitrogen source (ammonium chloride, NH_4Cl) was made with ^{15}N instead of ^{14}N . As a result, all the DNA in the bacteria was "heavy." They grew another culture in a medium with ^{14}N , and extracted DNA from both cultures. When the extracts were combined and centrifuged with CsCl, two separate DNA bands formed, showing that this method could distinguish DNA samples of slightly different densities.

Next, Meselson and Stahl grew another *E. coli* culture on ^{15}N medium, then transferred it to normal ^{14}N medium and allowed the bacteria to continue growing (Figure 11.10). Under the conditions they used, *E. coli* replicates its DNA every 20 minutes. Meselson and Stahl collected some of the

7.70 The Meselson-Stahl Experiment

Density gradient centrifugation revealed a pattern that supports the semiconservative model of DNA replication.

EXPERIMENT

Question: Does DNA replicate semiconservatively, or by some other mechanism?

METHOD

f

Grow bacteria in ^{15}N (heavy) medium.

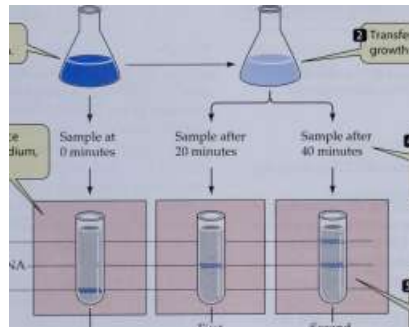
§J Before the bacteria reproduce the first time in the light medium, all DNA (parental) is heavy.

RESULTS

14;

N/ 14 N (light) DNA

14 N/15N (intermediate) DNA 15N/15N (heavy) DNA



Parental (all heavy)

INTERPRETATION

'y'^ss^

Transfer to 14 N (light) medium; growth continues.

Samples are taken after 20 minutes (the time one round of duplication takes) and 40 minutes (two rounds of duplication).

First

generation

(intermediate)

J

'Ni^QtFWStf^y •

Second

generation

(half are all light)

^x^^T&Ky^/

Parental New strand strand

N

15N

14

' / \tf^>SWW\ /

-^Xy^y^y^Ay^,

If each strand served as a template for the second strand, DNA of the first generation would be of an intermediate density, and half the DNA from the second generation would be intermediate and half light. This is what was in fact observed.

Conclusion: DNA replication is semiconservative.

208 CHAPTER ELEVEN

Growing strand

1

Template strand

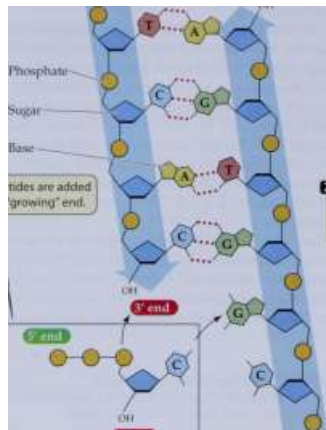
Growing strand

OH

Phosphate

QNucl Ttotr

Nucleotides are added to the "growing" end.



The enzyme DNA polymerase III adds the next deoxyribonucleotide, with the base C, to the —OH group at the 3' end of the growing strand.

$C \wedge > \wedge ?$

dCTP

S

Pyrophosphate ion

flHift

| Bonds linking the phosphate groups are broken, releasing energy to drive the reaction.

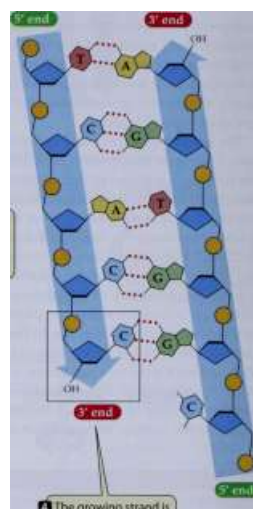


\

O

O

Phosphate ions



I The growing strand is antiparallel to the template strand.

11.11 Each New DNA Strand Grows from its 5' End to its 3' End

A DNA strand, with its 3' end at the top and its 5' end at the bottom, is the template for the synthesis of the complementary strand at the left.

bacteria after each division and extracted DNA from the samples.

Meselson and Stahl observed that the DNA banding in the density gradient was different in each bacterial generation. At the time of the transfer, the DNA was uniformly labeled with ^{15}N , and hence was relatively dense. After one generation, when the DNA had been duplicated once, all the DNA was of an intermediate density. After two generations, there were two equally large DNA bands: one of low density and one of intermediate density. In samples from subsequent generations, the proportion of low-density DNA increased steadily.

The results of this experiment can be explained by the semiconservative model of DNA replication. The high-density DNA had two ^{15}N strands; the intermediate-density DNA had one ^{15}N and one ^{14}N strand; and the low-density DNA had two ^{14}N strands. In the first round of DNA replication, the strands of the double helix—both heavy with ^{15}N —separated. While separated, each strand

acted as the template for a second strand, which contained only ^{14}N and hence was less dense. Each double helix then consisted of one ^{15}N and one ^{14}N strand and was of intermediate density. In the second replication, the ^{14}N -containing strands directed the synthesis of partners with ^{14}N , creating low-density DNA, and the ^{15}N strands got new ^{14}N partners (see Figure 11.10).

The crucial observation demonstrating the semiconservative model was that intermediate-density DNA ($^{15}\text{N}^{14}\text{N}$) appeared in the first generation and continued to appear in subsequent generations. With the other models, the results would have been quite different. In the conservative model, the first generation would have had both high-density DNA ($^{15}\text{N}^{15}\text{N}$) and low density DNA ($^{14}\text{N}^{14}\text{N}$), but no intermediate DNA. In the dispersive model, the density of the new DNA would have been between low and high, and not exactly intermediate.

Soon after Meselson and Stahl published their work, other scientists showed that semiconservative replication occurred in the DNAs of eukaryotic plant and animal cells. Using labeled DNA, they even demonstrated that chromatids appeared to replicate semiconservatively, providing the first evidence that a chromatid is a single molecule of double-helical DNA.



The Mechanism of DNA Replication

But how does DNA get replicated semiconservatively? There are four requirements for this process:

- ▶ DNA must act as a template for complementary base pairing.
- ▶ The four deoxyribonucleoside triphosphates, dATP, dGTP, dCTP, and dTTP, must be present.
- ▶ A DNA polymerase enzyme is needed to bring the substrates to the template and catalyze the reactions.
- ▶ A source of chemical energy is needed to drive this highly endergonic synthesis reaction.

DNA replication takes place in two steps:

- ▶ The DNA is locally denatured (unwound) to separate the two template strands and make them available for base pairing.
- ▶ The new nucleotides are linked by covalent bonding to each growing strand in a sequence determined by complementary base pairing.

A key observation of virtually all DNA replication is that nucleotides are always added to the growing strand at the 3' end—the end at which the DNA strand has a free hydroxyl ($-\text{OH}$) group on the 3' carbon of its terminal deoxyribose (Figure 11.11). The three phosphate groups in a deoxyribonucleoside triphosphate are attached to the 5' position of the sugar (see Figure 11.7). So when a new nucleotide is added to DNA, it can attach only to the 3' end.

When DNA polymerase brings a new deoxyribonucleoside triphosphate to the 3' end of a growing chain, the free hydroxyl group on the chain reacts with one of the substrate's phosphate groups. As this happens, the bond linking the terminal two phosphate groups to the rest of the deoxyribonucleoside triphosphate breaks, and thereby releases energy for this reaction. The resulting pyrophosphate ion, consisting of the two terminal phosphate groups, also breaks, forming two phosphate ions and in the process releasing additional free energy. The phosphate group still on the nucleotide becomes part of the sugar-phosphate backbone of the growing DNA molecule.

DNA is threaded through a replication complex

DNA is replicated through the interaction of the template DNA with a huge protein complex that catalyzes the reactions. All chromosomes have at least one sequence of nucleotides, called the origin of replication, that is recognized by this replication complex. DNA replicates in both directions from the origin, forming two replication forks. Both of the separated strands of the parent molecule act as templates, and the formation of the new strands is guided by complementary base pairing.

Until recently, DNA replication was depicted as a locomotive (the replication complex) moving along a railroad track (the DNA) (Figure 11.12a). The current view is that this is not so. Instead, the replication complex is stationary,

attached to nuclear structures, and it is the DNA that moves, essentially threading through the complex as single strands and emerging as double strands (Figure 11.12b). During S phase in eukaryotes, there are about 100 replication complexes, and each of them contains as many as 300 individual replication forks.

All replication complexes contain several proteins with different roles. We will describe these proteins as we examine the

steps of the process:

- ▶ DNA helicase opens up the double helix.
- ▶ Single-strand binding proteins keep the two strands separated.
- ▶ RNA primase makes the primer strand needed to get replication under way.
- ▶ DNA polymerase adds nucleotides to the primer that are complementary to the template, proofreads the DNA, and repairs it.
- ▶ DNA ligase seals up breaks in the sugar-phosphate backbone.

Small, circular DNAs replicate from a single origin, while large, linear DNA's have many origins

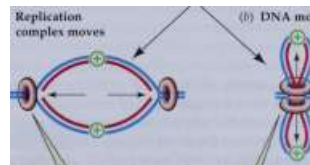
The key event at the origin of replication is the localized unwinding (denaturation) of DNA. There are several forces that hold the two strands together, including hydrogen bonding and the hydrophobic stacking of bases. An enzyme, DNA helicase, uses energy from ATP hydrolysis to unwind the DNA, and special proteins bind to the unwound strands to keep them that way, preventing them from reassociating into a double helix. This makes the two template strands available for complementary base pairing.

Parent DNA Origin of

strands replication Replication complex

(a) Replication complex moves

(b) DNA moves



Two replication complexes move apart as the DNA replicates in two directions away from the origin.

The replication complexes are stationary and the DNA threads through.

7 7.72 Two Views of DNA Replication

(a) It was once thought that the replication complex moved along DNA. (b) Newer evidence suggests that the DNA is threaded through the stationary complex.

210 CHAPTER ELEVEN

(n) Circular chromosome

The origin of replication binds to the replication complex.

Replication complex

DNA is spooled through

the complex, and

comes out replicated. li^

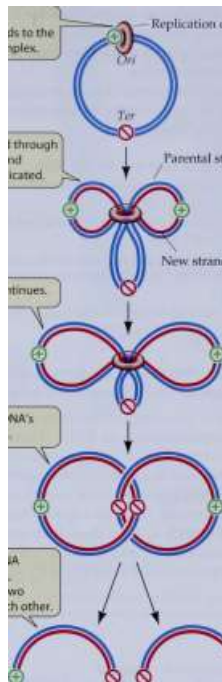
Parental strand

I Replication continues.

f

The two new DNA's are interlocked.

o An enzyme, DNA topoisomerase, separates the two DNA's from each other.



WW

(b) Linear chromosome

There are many origins of DNA replication.

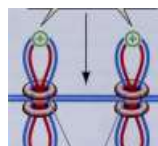


Origin of replication

11.13 Replication in Small Circular and Large Linear Chromosomes

(a) Small chromosomes have a single origin of replication and are circular, (b) Larger linear chromosomes have many origins of DNA replication.

£| DNA is replicated from several origins simultaneously.



Replication forks

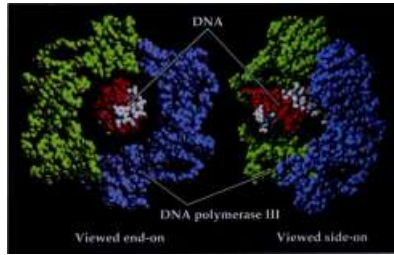
Small chromosomes, such as the 3-million-base-pair DNA of bacteria, have a single origin of replication. As the DNA moves through the replication complex, the replication forks grow around the circle (Figure 11.13a). Finally, two interlocking circular DNA's are formed, and they are separated by an enzyme called DNA topoisomerase.

In large chromosomes, such as a human chromosome that has 80 million base pairs, there are hundreds of origins of replication. Because each replicating factory has many adjacent replication complexes, adjacent origins of replication along the linear chromosome can bind at the same time and are replicated simultaneously. So there are many replication forks in eukaryotic DNA (Figure 11.13b).

DNA polymerases need a primer

Like most enzymes, DNA polymerases are much larger than their substrates, the deoxyribonucleoside triphosphates, and the template DNA, which is very thin. Molecular models of the enzyme-substrate-template complex (Figure 11.14) show that the enzyme is shaped like an open hand with a palm, a thumb, and fingers. The palm holds the active site of the enzyme and brings together the substrate and template. The finger regions rotate inward and have precise shapes that just fit the appropriate nucleotide. DNA polymerases can elongate a polynucleotide strand, but they cannot start a strand from scratch. DNA polymerases require the assistance of a previously existing strand of DNA or RNA to which they can add new nucleotides. Such a helper strand is called a primer. DNA polymerases add nucleotides to the 3' end of the primer.

In DNA replication, the primer is a short single strand of RNA (Figure 11.15). This RNA strand, complementary to the DNA template strand, is formed by



The enzyme is much larger than the DNA. It is shaped like a hand, and in side view, "fingers" curl around DNA and can recognize the different shapes of the four bases.

I Primase binds to DNA and synthesizes an RNA primer.

```
iiii^iiijooooooooooooooooooooooooooooo
```

polymerase III

3 Primase is released.

© nnnrïnnnnnnnninTnnTnnnninTnnnni ©

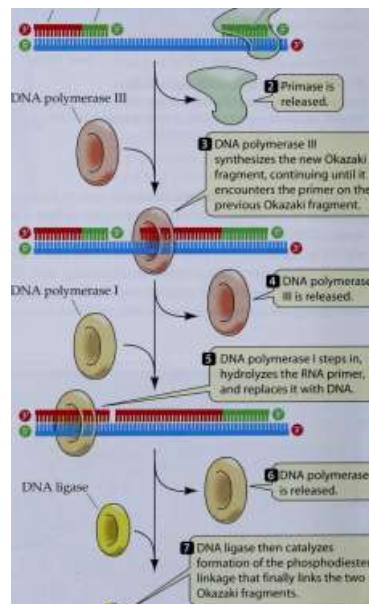
//

11.15 No DNA Forms without a Primer

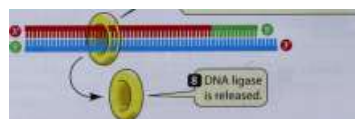
an enzyme called a primase, which is one of several polypeptides bound together in an aggregate called a primosome. DNA polymerase adds nucleotides to the primer until the replication of that section of DNA has been completed. Then the RNA primer is degraded and DNA is added in its place. When DNA replication is complete, each daughter molecule consists only of DNA.

Synthesis of the eading strand is continuous.

Lagging RNA primer RNA primase^ RNA primer strand / \, -/v



DNA ligase then catalyzes formation of the phosphodiester linkage that finally links the two Okazaki fragments.



Fortunately, our cells normally have at least three DNA repair mechanisms at their disposal:

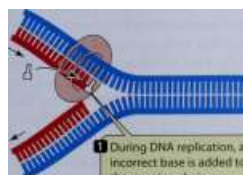
- ▶ A proofreading mechanism corrects errors as DNA polymerase makes them.
- ▶ A mismatch repair mechanism scans DNA after it has been made and corrects any base-pairing mismatches.
- ▶ An excision repair mechanism removes abnormal bases that have formed because of chemical damage and replaces them with functional bases.

Proofreading and repair mechanisms ensure that DNA replication is accurate

After introducing a new nucleotide into a growing polynucleotide strand, the DNA polymerases perform a proofreading function (Figure 11.19a). When a DNA polymerase recognizes a mispairing of bases, it removes the improperly introduced nucleotide and tries again. (DNA helicase, DNA ligase, and other proteins of the replication complex also play roles in this key mechanism.) The polymerase is usually successful in inserting the correct monomer the second time around. As a result of this proofreading mechanism, the overall error rate for DNA replication is greatly lowered: Starting from an initial error rate of 1 base in every 10^6 , the final error rate is only about 1 base in every 10^9 bases replicated.

After DNA has been replicated and during genetic recombination, a second mechanism surveys the newly repli-

(a) DNA proofreading



UU miHimumi

I During DNA replication, an incorrect base is added to the growing chain.

cated molecule and looks for mismatched base pairs (Figure 11.19b). For example, this mismatch repair system might detect an AC base pair instead of an AT pair. Since both AT and GC pairs obey the base-pairing rules, how does the repair mechanism "know" whether the AC pair should be repaired by removing the C and replacing it with T, for instance, or by removing the A and replacing it with G?

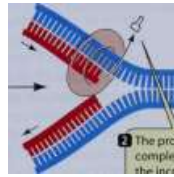
The repair mechanism can detect the "wrong" base because a newly synthesized DNA strand is chemically modified some time after replication. In eukaryotes, methyl groups ($-\text{CH}_3$) are added to some cytosines to form 5-methylcytosine. In prokaryotes, guanine is methylated. Right after replication, methylation has not yet occurred, so the newly replicated strand is "marked" by being unmethylated, as the one in which errors should be corrected. When mismatch repair fails, DNA sequences are altered. One form of colon cancer arises in part from a failure of mismatch repair.

DNA molecules can also be damaged during the life of a cell (e.g., when it is in G1). Some cells live and play important roles in

the organism for many years, even though their DNA is constantly at risk from hazards such as high-energy radiation, chemicals that induce mutations, and random spontaneous chemical reactions. Cells owe their lives

7.7.9 DNA Repair Mechanisms

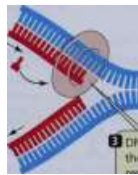
The proteins of DNA replication also play roles in the life-preserving DNA repair mechanisms, helping to ensure the exact replication of template DNA.



mmmmnnnn

iiiiiiiiUU

I The proteins of the replication complex immediately excise the incorrect base.



iiiiiiiiiiii

I DNA polymerase adds the correct base and replication proceeds.

(b) Mismatch repair

iluuuuui^Buuiiiiii.

t During DNA replication, a base was mispaired.

T

^

iiiiiiii'iiiiiiii

iiimiiipimiiiM

The mismatch repair proteins excise the mismatched base.

f

DNA polymerase adds the correct base

innnfflpfni

(c) Excision repair

iiiiiiiiijUiiiiiiiiii

t

A base in DNA is damaged so that it is not functional.

HThe

r moif

imTTTTT iiiiiiiiii

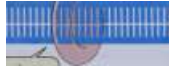
imiuiip iiiuiML

excision repair proteins

excise the damaged base and some adjacent bases.

nnn

o DNA polymerase adds the correct bases by 5'-to-3' replication of the short strand.



_ ^1

214 CHAPTER ELEVEN

to DNA repair mechanisms. For example, in excision repair, certain enzymes "inspect" the cell's DNA (Figure 11.19c). When they find mispaired bases, chemically modified bases, or points at which one strand has more bases than the other (with the result that one or more bases of one strand form an unpaired loop), these enzymes cut the defective strand. Another enzyme cuts away the bases adjacent to and including the offending base, and DNA polymerase and DNA ligase synthesize and seal up a new (usually correct) piece to replace the excised one.

Our dependence on this repair mechanism is underscored by our susceptibility to various diseases that arise from excision repair defects. One example is the skin disease xeroderma pigmentosum. People with this disease lack a mechanism that normally repairs damage caused by the ultraviolet radiation in sunlight. Without this mechanism, a person exposed to sunlight develops skin cancer.

DNA repair requires energy

What does it cost the cell to keep its DNA accurate and ensure that it replicates properly? At first glance, you might expect DNA polymerization to be fairly "neutral" energetically: Adding a new monomer to the chain requires the formation of a new phosphodiester bond, but is supported by the hydrolysis of one of the high-energy bonds in the de-oxyribonucleoside triphosphate (see Figure 11.11). Overall, however, this reaction is slightly endergonic. But help is available in the form of the pyrophosphate ion released in the polymerization reaction. The enzyme pyrophosphatase cleaves the high-energy bond in the pyrophosphate. Coupling this reaction to the polymerization gives it a big boost.

Noncovalent bonds also play a major role in favoring DNA polymerization. Hydrogen bonds form between the complementarily paired bases, and other weak interactions form as the bases stack in the middle of the double helix. These bonds and interactions stabilize the DNA molecule and help drive the polymerization reaction. Thus DNA synthesis itself does not take a tremendous toll in energy.

DNA repair processes, however, are far from cheap energetically. Some are very inefficient. Nonetheless, the cell deploys many DNA repair mechanisms, some overlapping in function with others. Why? Perhaps because the cell simply can't afford to leave its genetic information unprotected, regardless of the cost.

Practical Applications of DNA Replication

The principles that underlie DNA replication in cells have been applied to develop two laboratory techniques that have been vital in analyzing genes and genomes. First, the nucleotide sequence of a DNA molecule can be determined, and second, short DNA sequences can be copied using the polymerase chain reaction technique.

The nucleotide sequence of DNA can be determined

As we saw earlier in this chapter, the deoxyribonucleoside triphosphates (dNTP's) that are the normal substrates for DNA replication contain the sugar 2-deoxyribose. If instead of this, the sugar used is 2,3-dzdeoxyribose, the resulting nucleoside triphosphates (ddNTP's) will still be picked up by DNA polymerase and added to a growing DNA chain. However, because ddNTP's lack a hydroxyl group at the 3' position, the next nucleotide cannot be added (Figure 11.20fl). Thus synthesis stops at the place where ddNTP is incorporated into the growing end of a DNA strand.

In the sequencing technique, a molecule of DNA (usually no more than 500 base pairs long) is denatured. The resulting single-strands of DNA are mixed with:

- ▶ DNA polymerase to synthesize the complementary strand,
- ▶ short primers appropriate for that sequence,
- ▶ the four dNTPs (dATP, dGTP, dCTP, and dTTP), and
- ▶ small amounts of the four ddNTPs, each with a fluorescent "tag" that emits a different color of light.

The reaction mixture soon contains mostly a DNA mixture of the template DNA strands and shorter, new complementary strands. The latter, each ending with a ddNTP, are of varying lengths. For example, each time a T is reached on the template strand, the growing complementary strand adds either dATP or ddATP. If dATP is added, the strand continues to grow. If ddATP is added, chain growth stops.

After DNA replication has been allowed to proceed for a while in a test tube, the numerous short fragments are denatured from their templates and separated by electrophoresis (see Figure 17.2). This technique measures the length of the DNA fragments, and can detect differences in fragment length as short as one base in 500. During the electrophoresis run, the fragments pass through a laser beam that excites the fluorescent tags. The light emitted is then detected, and the resulting

information—that is, which ddNTP is at the end of a strand of which length—is fed into a computer, which processes it and prints out the sequence (Figure 11.20b). The computer can also be programmed to analyze the sequence, and these analyses have formed the basis of the new science of genomics, as we will describe in Chapters 13, 14, and 18.

The polymerase chain reaction makes multiple copies of DNA

Since DNA can be replicated in the test tube, using enzymes from *E. coli* and simple substrates, it is possible to make quantities of a single DNA sequence. The polymerase chain reaction (PCR) technique is an extension of this early work, which essentially automates the process and makes it much more efficient. PCR is not very complicated: A short

RESEARCH METHOD

(a)

O

o

O

Base

(A, U, G, or C)

-O — P—O— P — O— P — O — CH

I I I

o- o- o-

Ribonucleoside triphosphate (NTP)

O.



O

o

O

Base

(A, T, G, or C)

-O — P—O— P — O— P — O — CH, ~

I I I

o- o- o-

N' H

HO OH

Deoxyribonucleoside H triphosphate (dNTP)



O O O

II II II

O — P—O— P — O— P — O — CH,

Base

(A, T, G, or C)

I o-

I O"

I

O"

HO H

Dideoxynucleoside triphosphate (ddNTP)

For a DNA fragment to be sequenced, it must be labeled with a fluorescent dye.

for which the base sequence is to be determined (the template).



<

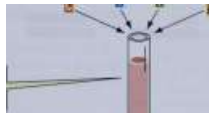
Each of the 4 ddNTP's is bound to a fluorescent dye.



Absence of OH at 3' position means that additional nucleotides cannot be added.

ddCTP ddGTP ddTTP ddATP

A sample of this unknown DNA is combined with primer, DNA polymerase, 4 dNTP's, and the fluorescent ddNTP's. Synthesis begins.



W

The results are illustrated here by what binds to a T in the unknown strand. If ddATP is picked up, synthesis stops. A series of fragments of different lengths is made, each ending with a ddATP.

Template strand

HE



©M1 —

©

ATCTGGGCTATTCGGGCGT

t

The newly synthesized strands of various lengths are separated by electrophoresis.

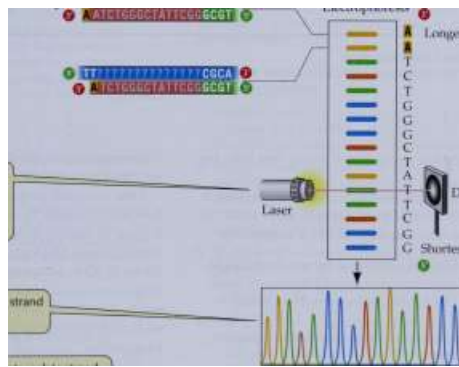
-Primer (sequence known)

Electrophoresis

Each strand fluoresces a color that identifies the ddNTP that terminated the strand. The color at the end of each fragment is detected by a laser beam.

The sequence of the newly synthesized strand of DNA can now be deduced...

...and converted to the sequence of the template strand.



Longest fragment

Detector

Shortest fragment ©



©AATCTGGGCTATTCGG©

si TTAGACCCGATAAGCCCGCA

©

7.20 Sequencing DNA

(a) The normal substrates for DNA replication are dNTP's. The slightly different structure of ddNTP's can cause DNA synthesis to stop, (b) When labeled ddNTP's are incorporated into a mixture containing a single-stranded DNA template of unknown sequence, the result is an electrophoresis of fragments of varying lengths.

216 CHAPTER ELEVEN

RESEARCH METHOD

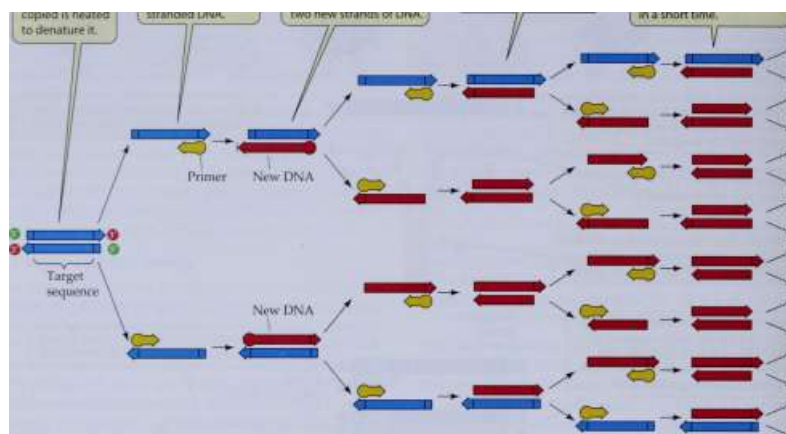
| A DNA molecule with a target sequence to be copied is heated to denature it.

Q When the mixture cools, primers bond to the single-stranded DNA.

Q Primers, dNTP's, and DNA polymerase are added to synthesize two new strands of DNA.

Q The process is repeated, doubling the amount of DNA.

o By repeating the process, many copies of the original DNA can be produced in a short time.



7.2.7 The Polymerase Chain Reaction

The steps in this cyclic process are repeated many times to produce multiple copies of a DNA sequence.

region of DNA is copied many times in the test tube by DNA polymerase.

PCR is a cyclic process in which the following sequence of steps is repeated over and over again (Figure 11.21). It begins with the same first two steps as DNA sequencing:

► Double-stranded DNA is denatured by heat into single strands.

- Short primers for DNA replication are added to the mixture.
- DNA polymerase catalyzes the production of complementary new strands.

A single cycle, taking a few minutes, doubles the amount of DNA and leaves the new DNA in the double-stranded state. Repeating the cycle many times can theoretically lead to a geometric increase in the number of copies of the DNA sequence.

The PCR technique requires that the base sequences at the 3' end of each strand of the target DNA be known so that a complementary primer, usually 15 to 20 bases long, can be made in the laboratory. Because of the uniqueness of DNA sequences, usually two primers of this length will bind to only one region of DNA in an organism's genome.

This specificity in the face of the incredible diversity of target DNA is a key to the power of PCR.

One potential problem with PCR involves its temperature requirements. To denature the DNA during each cycle, it must be heated to more than 90°C—a temperature that destroys most DNA polymerases. Then it must be cooled to about 55°C to allow the primer to hydrogen-bond to the single strands of template DNA. The PCR method would not be practical if new polymerase had to be added during each cycle after denaturation—an expensive and laborious proposition.

This problem was solved by nature: In the hot springs at Yellowstone National Park, as well as other locations, there live bacteria called, appropriately, *Thermus aquaticus*. The means by which these organisms survive temperatures up to 95°C was investigated by bacteriologist Thomas Brock and his colleagues. They discovered that *T. aquaticus* has an entire metabolic machinery that is heat-resistant, including DNA polymerase that does not denature at this high temperature.

Scientists pondering the problem of amplifying DNA by PCR read Brock's basic research articles and got a clever idea: Why not use *T. aquaticus* DNA polymerase in the PCR

DNA AND ITS ROLE IN HEREDITY 217

reaction? It would not be denatured, and thus would not have to be added during each cycle. The idea worked, and earned biochemist Kerry Mullis a Nobel prize. PCR has had an enormous impact on genetic research. Some of its most striking applications will be described in Chapters 13 through 17.

Chapter Summary

DNA: The Genetic Material

- In addition to circumstantial evidence (the location and quantity of DNA in the cell), two experiments provided a convincing demonstration that DNA is the genetic material.
- In one experiment, DNA from a virulent strain of pneumo-coccus bacteria genetically transformed nonvirulent bacteria into virulent bacteria. Review Figure 11.1
- In a second set of experiments, labeled viruses were incubated with host bacteria. Labeled viral DNA entered the host cells, where it produced hundreds of new viruses bearing the label. Review Figures 11.2, 11.3

The Structure of DNA

- X-ray crystallography showed that the DNA molecule is a helix. Review Figure 11.4
- DNA is composed of nucleotides, each containing one of four bases—adenine, cytosine, thymine, or guanine. Biochemical analysis revealed that the amount of adenine equals the amount of thymine and the amount of guanine equals the amount of cytosine. Review Figure 11.5
- Putting the accumulated data together, Watson and Crick built a model of the DNA molecule. They proposed that DNA is a double-stranded helix in which the strands are antiparallel and the bases are held together by hydrogen bonding. This model accounts for the genetic information, mutation, and replication functions of DNA. Review Figures 11.6, 11.7

DNA Replication

- Three possible models for DNA replication were hypothesized: semiconservative, conservative, and dispersive. Review Figure 11.8
- An experiment by Meselson and Stahl proved the replication of DNA to be semiconservative. Each parent strand acts as a template for the synthesis of a new strand; thus the two replicated DNA helices contain one parent strand and one newly synthesized strand each. Review Figures 11.9, 11.10

The Mechanism of DNA Replication

- In DNA replication, the enzyme DNA polymerase catalyzes the addition of nucleotides to the 3' end of each strand. Nucleotides are added by complementary base pairing with the template strand of DNA. The substrates are deoxyribonucleoside triphosphates, which are hydrolyzed as they are added to the growing chain, releasing energy that fuels the synthesis of DNA. Review Figure 11.11
- The DNA replication complex is in a fixed location and DNA is threaded through it for replication. Review Figure 11.12

- ▶ Many proteins assist in DNA replication. DNA helicases unwind the double helix, and the template strands are stabilized by other proteins.
- ▶ Prokaryotes have a single origin of replication; eukaryotes have many. Replication in both cases proceeds in both directions from an origin of replication. Review Figure 11.13
- ▶ An RNA primase catalyzes the synthesis of short RNA primers, to which nucleotides are added as the chain grows. Review Figure 11.15
- ▶ Through the action of DNA polymerase, the leading strand grows continuously in the 5'-to-3' direction until the replication of that section of DNA has been completed. Then the RNA primer is degraded and DNA is added in its place.
- ▶ On the lagging strand, which grows in the other direction, DNA is still made in the 5'-to-3' direction (away from the replication fork). But synthesis of the lagging strand is discontinuous: The DNA is added as short fragments to primers, then the polymerase skips past the 5' end to make the next fragment. Review Figures 11.16,11.17,11.18

DNA Proofreading and Repair

- ▶ The machinery of DNA replication makes about one error in 10^6 nucleotides bases added. These errors are repaired by three different mechanisms: proofreading, mismatch repair, and excision repair. DNA repair mechanisms lower the overall error rate of replication to about one base in 10^9 . Review Figure 11.19
- ▶ Although energetically costly and somewhat redundant, DNA repair is crucial to the survival of the cell.

Practical Applications of DNA Replication

- ▶ The principles of DNA replication can be used to determine the nucleotide sequence of DNA. Review Figure 11.20
- ▶ The polymerase chain reaction technique uses DNA polymerases to repeatedly replicate DNA in the test tube. Review Figure 11.21

For Discussion

1. Outline a series of experiments using radioactive isotopes to show that bacterial DNA and not protein enters the host cell and is responsible for bacterial transformation.
2. Suppose that Meselson and Stahl had continued their experiment on DNA replication for another ten bacterial generations. Would there still have been any $^{14}\text{N}^{15}\text{N}$ hybrid DNA present? Would it still have appeared in the centrifuge tube? Explain.
3. If DNA replication were conservative rather than semi-conservative, what results would Meselson and Stahl have observed? Diagram the results using the conventions of Figure 11.10.
4. Using the following information, calculate the number of origins of DNA replication on a human chromosome: DNA polymerase adds nucleotides at 3,000 base pairs per minute in one direction; replication is bidirectional; the S phase lasts 300 minutes; there are 120 million base pairs per chromosome. With a typical chromosome 3 cm long, how many origins are there per micrometer?
5. The drug dideoxycytidine (used to treat certain viral infections) is a nucleoside made with 2'-3'-dideoxyribose. This sugar lacks —OH groups at both the 2' and the 3' positions. Explain why this drug would stop the growth of a DNA chain if it was added to DNA.



From DNA to Protein: Genotype to Phenotype



The ocean is literally alive with light. About 90 percent of all marine animals living at depths between 200 and 1,000 meters are bioluminescent—they can use chemical reactions to produce light. In some cases, it helps them find a mate or food. In others, it acts like a burglar alarm, startling a predator. The jellyfish in the photograph is in the latter category. Clearly, this is a genetically transmitted characteristic that is important to the animal's survival.

This jellyfish creates light by means of two proteins acting in sequence. The first, aequorin, absorbs light and transmits some of its excitation energy to a second protein, green fluorescent protein (GFP). It is GFP that gives off a green glow. As you will see in Chapter 17, GFP has become an invaluable tool in biological research, as it can be attached to other molecules, allowing them to be observed in real time in living cells and tissues because of the green glow. Here, however, the important conclusion is that bioluminescence—a phenotype—is essentially produced by the actions of two proteins. And these proteins are coded for by DNA sequences.

This chapter deals with the mechanisms by which genes are expressed as proteins. We begin with evidence for the relationship between genes and proteins, and then fill in some of the details of the processes of transcription and translation. Finally, we define mutations and their phenotypes in specific molecular terms.

One Gene, One Polypeptide

There are many steps between genotype and phenotype. Genes cannot, all by themselves, directly produce a phenotypic result, such as a particular eye color, a specific seed shape, or a cleft chin, any more than a compact disk can play a symphony without the help of a CD player.

With the gene defined as a DNA sequence, the first step in relating genes to phenotypes was to define phenotypes in molecular terms. The molecular basis of phenotypes was actually discovered before the discovery of DNA as the genetic material. Using organisms as diverse as humans and bread molds, scientists studied the chemical differences between organisms carrying wild-type and mutant alleles, and that the major phenotypic differences were in specific proteins.

Light in the Depths of the Ocean

The bioluminescent Pacific jellyfish, *Aequorea victoria*, "lights up" when startled by a predator.

In the 1940s, a series of experiments by George W. Beadle and Edward L. Tatum at Stanford University showed that when an altered gene resulted in an altered phenotype, the latter showed up as an altered enzyme protein (Figure 12.1). This finding was critically important in defining the phenotype in chemical terms.

Beadle and Tatum experimented with the bread mold *Neurospora crassa*. The nuclei in the body of the mold are haploid (n), as are the reproductive spores. (This fact is important because it means that even recessive mutant alleles are easy to detect in experiments.) Beadle and Tatum grew *Neurospora* on a minimal nutritional medium of sucrose, minerals, and a vitamin. Using this medium, the enzymes of wild-type *Neurospora* could catalyze the metabolic reactions needed to make all the chemical constituents of their cells, including proteins. These wild-type strains are called prototrophs ("original eaters").

Beadle and Tatum treated wild-type *Neurospora* with X-rays, a mutagen (something known to cause mutations). When they examined the treated molds, they found some



EXPERIMENT

Question: What is the relationship between genes and enzymes in a biochemical pathway?

METHOD

Put spores of each mutant strain on a minimal medium (mm) with no supplements; mm + arginine; mm + citrulline; and mm + ornithine.

RESULTS

The wild type grows on all media; it can synthesize its own arginine.

Mutant strain 1 grows only when arginine is supplied.

This means it lacks the ability to convert either citrulline or ornithine to arginine.

Mutant strain 2 grows on either arginine or citrulline, but not on ornithine.

This means it can convert citrulline to arginine, but cannot convert ornithine.

Mutant strain 3 does not grow on minimal medium, but grows when any one of the three supplements are added.

This means it can convert ornithine to citrulline and citrulline to arginine.

INTERPRETATION

If an organism cannot convert one particular compound to another, it presumably lacks an enzyme required for the conversion, and the mutation is in the gene that codes for that enzyme.

Conclusion: The synthesis of arginine proceeds like this...

...and each gene specifies a particular enzyme.

All the mutant strains grow if the amino acid arginine is added (indeed, we selected the strains because they require arginine).



Strain

Wild type



Supplements to minimal medium

None

Ornithine

%

Citrulline

c

%

%

Arginine

%

%

Strain 3 is blocked at this step.

Strain 2 is blocked at this step.

Strain 1 is blocked at this step.

Precursor J^jE^aJ^aOrnithine

"t t

EfcCitrulline

Arginine

t

Gene A

GeneB

GeneC

12.1 One Gene, One Enzyme

Beadle and Tatum studied several auxotrophic mutants of Neurospora. Different auxotrophic mutants required the addition of different

ent nutrients in order to obtain the arginine required for their growth. Step through the figure to follow the reasoning that upheld the "one-gene, one-enzyme" hypothesis.

220 CHAPTER TWELVE

mutant strains could no longer grow on minimal medium, but needed additional nutrients that they could not make on their own. The scientists proposed that these auxotrophs ("increased eaters") must have suffered mutations in genes that coded for enzymes necessary for the synthesis of the nutrients they now needed to ingest. For each auxotrophic strain, Beadle and Tatum were able to find a single compound that, when added to the minimal medium, supported the growth of that strain. This result supported the idea that mutations have simple effects, and that each mutation causes a defect in only one enzyme in a metabolic pathway.

One group of auxotrophs, for example, could grow on minimal medium supplemented with the amino acid arginine. (Wild-type *Neurospora* makes arginine by itself.) These mutant strains were classified as arg mutants. Were their mutations different alleles of the same gene, or were they in different genes, each coding for an enzyme along the biochemical pathway for arginine synthesis? Mapping studies established that some of the arg mutations were at different loci, or on different chromosomes, and so were not alleles. Beadle and Tatum concluded that different genes could participate in governing a single biosynthetic pathway—in this case, the pathway leading to arginine synthesis.

By growing different arg mutants in the presence of various compounds suspected to be intermediates in the synthetic metabolic pathway for arginine, Beadle and Tatum were able to classify each mutation as affecting one enzyme or another, and to order the compounds on the pathway (see Figure 12.1). Then they broke open the wild-type and mutant cells and examined them for enzyme activities. The results confirmed their hypothesis: Each mutant strain was indeed missing a single active enzyme in the pathway.

If a genetic mutation results in an abnormal or missing enzyme, then the wild-type gene must code for the normal enzyme. This conclusion led Beadle and Tatum to formulate the one-gene, one-enzyme hypothesis. According to this hypothesis, the function of a gene is to control the production of a single, specific enzyme. This proposal strongly influenced the subsequent development of the sciences of genetics and molecular biology.

The English physician Archibald Garrod, who studied the inherited disease alkaptonuria, had made this proposal 40 years before. He linked the biochemical phenotype of the disease to an abnormal gene and a missing enzyme. There are hundreds of examples of such hereditary diseases, and we will return to them in Chapter 18.

The gene-enzyme relationship requires modification when we consider that many enzymes are composed of more than one polypeptide chain, or subunit (that is, they have a quaternary structure). In this case, each polypeptide chain is specified by its own separate gene. Thus, it is more correct to speak of a one-gene, one-polypeptide hypothesis: The function of a gene is to control the production of a single, specific polypeptide.

Much later, it was discovered that some genes code for forms of RNA that do not become translated into polypep-

tides, and that still other genes are involved in controlling which other DNA sequences are expressed. While these discoveries have overthrown the idea that all genes code for proteins, they did not invalidate the relationship between genes and polypeptides.

DNA, RNA, and the Flow of Information

Now let us turn our attention to how a gene expresses itself as a polypeptide. This expression occurs in two steps. The first step, transcription, copies the information of a DNA sequence (the gene) into corresponding information in an RNA sequence. The second step, translation, converts this RNA information into an appropriate amino acid sequence in a polypeptide.

RNA differs from DNA

To understand the transcription and translation of genetic information, you need to understand the structure of RNA. RNA (ribonucleic acid) is a polynucleotide similar to DNA (see Figure 3.17), but it differs from DNA in three ways:

- ▶ RNA generally consists of only one polynucleotide strand [thus Chargaff's rule, $G = C$ and $A = T$ (see Figure 11.5), is usually true for DNA and not for RNA].
- ▶ The sugar molecule found in RNA is ribose, rather than the deoxyribose found in DNA.
- ▶ Although three of the nitrogenous bases (adenine, guanine, and cytosine) in RNA are identical to those in DNA, the fourth base in RNA is uracil (U), which is similar to thymine but lacks the methyl ($-\text{CH}_3$) group.

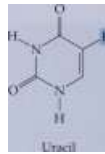
O

H

\

N

CH,



CT N'

I H

Thymine

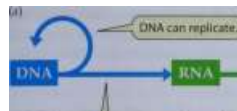
RNA can base-pair with single-stranded DNA, and this pairing obeys the same hydrogen-bonding rules as in DNA, except that adenine pairs with uracil instead of thymine. RNA can also fold over and base-pair with itself, as we will see with tRNA later in this chapter.

Information flows in one direction when genes are expressed

Francis Crick (of the Watson-Crick model) proposed what he called the central dogma of molecular biology. The central dogma is, simply, that DNA codes for the production of RNA (transcription), RNA codes for the production of protein (translation), and protein does not code for the production of protein, RNA, or DNA (Figure 2.2a). In Crick's words, "once 'information' has passed into protein it cannot get out again."

'3*

FROM DNA TO PROTEIN: GENOTYPE TO PHENOTYPE 221



Protein

Information coded in the sequence of base pairs in DNA is passed to molecules of RNA.

Information in RNA is passed to proteins. It never passes from proteins to nucleic acids.

(b)

ao^

J32J.

Protein

The reproductive cycle of retroviruses adds a step: reverse transcription.

72.2 The Central Dogma

(a) Information flows from DNA to proteins, as indicated by the arrows. In certain viruses, RNA can replicate to RNA. (b) The reproductive cycle of retroviruses adds a step, reverse transcription, to the central dogma.

The central dogma posed two questions:

- How does genetic information get from the nucleus to the cytoplasm? (As you know, most of the DNA of a eukaryotic cell is confined to the nucleus, but proteins are synthesized in the cytoplasm.)
- What is the relationship between a specific nucleotide sequence (in DNA) and a specific amino acid sequence (in a protein)?

To answer the first question, Crick and his colleagues developed the messenger hypothesis, according to which an RNA molecule forms as a complementary copy of one DNA strand of a particular gene. The process by which this RNA forms is called transcription. If each such RNA molecule contains the information from a gene, there should be as many different kinds of RNA molecules as there are genes. This messenger RNA, or mRNA, then travels from the nucleus to the cytoplasm, where it serves as a template for the synthesis of proteins.

To answer the second question, Crick proposed the adapter hypothesis: there must be an adapter molecule that can bind a specific amino acid at one end and recognize a sequence of nucleotides with another region. In due course, these adapters, called transfer RNA, or tRNA, were identified. Because they recognize the genetic message of mRNA and simultaneously carry specific amino acids, tRNA's can translate the language of DNA into the language of proteins. The tRNA adapters line up on the mRNA so that the amino acids are in the proper sequence for a growing polypeptide chain—a process called translation (Figure 12.3).

Summarizing the main features of the central dogma, the messenger hypothesis, and the adapter hypothesis, we

may say that a given gene is transcribed to produce a messenger RNA (mRNA) complementary to one of the DNA strands, and that transfer RNA (tRNA) molecules translate the sequence of bases in the mRNA into the appropriate sequence of amino acids.

RNA viruses modify the central dogma

According to the central dogma, DNA codes for RNA, and RNA codes for protein. All cellular organisms have DNA as their hereditary material. Only among viruses (and certain DNA sequences) are variations on the central dogma found.

Many viruses, such as the tobacco mosaic virus, influenza virus, and poliovirus, have RNA rather than DNA as their genetic material. With its nucleotide sequence, RNA could potentially act as an information carrier and be expressed as proteins. But since RNA is usually single-stranded, its replication is a problem. The viruses generally solve this problem by transcribing from RNA to RNA, making an RNA strand that is complementary to the genome. This "opposite" strand is used to make to make more copies of the genome by transcription.

HIV and certain tumor viruses also have RNA as their genome, but do not replicate it as RNA-to-RNA. Instead, after infecting a host cell, they make a DNA copy of their genome, and use it to make more RNA. This RNA is then used both as genomes for more copies of the virus and as mRNA (see Figure 12.2b). Synthesis of DNA from RNA is called reverse transcription. Not surprisingly, such viruses are called retroviruses. We will examine both types of RNA viruses in detail in the next chapter.

•ir ■i.iKSvm,,,...;;; UMr '». «' '»*

iiiiiiiiiiiiiiiiin

mRNA

If See Figure

rmation Transcription

(RNA synthesis)

I

ii iiiiii ii i iiiii ii iii i iiiiii nni iiiii i i i i iiiii iii



See Figures 12.9-12.12.

mnnnnnnn

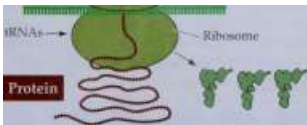
tRNAs —

Information

1

Translation

(protein synthesis)



12.3 From Gene to Protein

This figure summarizes the processes of gene expression in pro-karyotes. In eukaryotes, the processes are somewhat more complex.

222 CHAPTER TWELVE

Transcription: DNA-Directed RNA Synthesis

Transcription—the formation of a specific RNA from a specific DNA, requires the enzyme RNA polymerase. It also requires the appropriate ribonucleoside triphosphates (ATP, GTP, CTP, and UTP) and a DNA template. Within each gene, only otic of the strands—the template strand —is transcribed. The other, complementary DNA strand remains untranscribed. For different genes in the same DNA molecule, different strands may be transcribed. That is, the strand that is the

complementary strand in one gene may be the template strand in another.

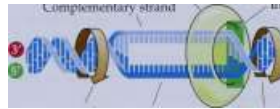
Not only mRNA is produced by transcription. The same process is responsible for the synthesis of tRNA and ribosomal RNA (rRNA), which constitutes a major fraction of the ribosomes. Like mRNA, these other forms of RNA are encoded by specific genes.

In DNA replication, as we know, the two strands of the parent molecule unwind, and each strand serves as the

Initiation

Q RNA polymerase binds to the promoter and starts to unwind the DNA strands.

RNA polymerase, Complementary strand



Initiation site

template for a new strand. In transcription, DNA partly unwinds so that it can serve as a template for RNA synthesis. As the RNA transcript forms, it peels away, allowing the DNA that has already been transcribed to be rewound into the double helix (Figure 12.4).

Transcription can be divided into three distinct processes: initiation, elongation, and termination. Let's consider each of these in turn.

Initiation of transcription requires a promoter and an RNA polymerase

The transcription of a gene begins at a promoter, a special sequence of DNA to which RNA polymerase binds very tightly. There is at least one promoter for each gene (or, in prokaryotes, each set of genes) to be transcribed into

^ 72.4 DNA Is Transcribed into RNA

DNA partially unwinds to serve as a template for RNA synthesis. The RNA transcript forms and then peels away, allowing the DNA that has already been transcribed to rewind into a double helix. Three distinct processes—initiation, elongation, and termination—constitute DNA transcription. RNA polymerase is much larger in reality than indicated here, covering about 50 base pairs.

Termination site



t+*\4*Yt*\#VK+1

/

Rewinding of DNA

Template strand

Unwinding of DNA

o RNA polymerase reads the DNA template strand from 3' to 5' and produces the RNA transcript from 5' to 3'.

Elongation

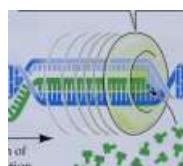
o(\

o

»\«\4Bt*\#i

Qimmmiiiiiii

Direction of transcription



ry^i!#yp#S^

Once RNA polymerase has bound to the promoter, it unwinds the DNA about 20 base pairs at a time and reads the template strand in the 3'-to-5' direction (see Figure 12.4). Like DNA polymerase, RNA polymerase adds new nucleotides to the 3' end of the growing strand. That is, the new RNA grows from its own 5' end to its 3' end. The RNA transcript is thus antiparallel to the DNA template strand.

We indicated in the previous chapter that DNA replication occurs at fixed locations in the nucleus, with the DNA moving through a replication complex. While there is some evidence that this may be the case in DNA transcription to RNA as well, it is not as convincing as for DNA replication,

so we will present the model of a "moving transcription complex" rather than a moving DNA molecule.

Transcription, like DNA replication, requires a lot of energy. Like replication, transcription draws on energy released by both the removal and the breakdown of the pyrophosphate group from each nucleotide added.

Unlike DNA polymerases, RNA polymerases do not inspect and correct their work. Transcription errors occur at a rate of one mistake for every 10^4 to 10^5 bases. Because many copies of RNA are made, these errors are not as potentially harmful as mutations in DNA.

Transcription terminates at particular base sequences

What tells RNA polymerase to stop adding nucleotides to a growing transcript? Just as initiation sites specify the start of transcription, particular base sequences in the DNA specify its termination. The mechanisms of termination are complex and of more than one kind. For some genes, the newly formed transcript simply falls away from the DNA template and the RNA polymerase. For others, a helper protein pulls the transcript away.

In prokaryotes, the translation of mRNA often begins (at the 5' end of the mRNA) before transcription of the mRNA molecule is complete. In eukaryotes, the situation is more complicated. First, there is a spatial separation of transcription (in the nucleus) and translation (in the cytoplasm). Second, the first product of transcription is a pre-mRNA that is longer than the final mRNA and must undergo considerable processing before it becomes the mRNA that can be translated. The advantages of this processing, and its mechanisms, will be discussed in Chapter 14.

The Genetic Code

The genetic code provides the specificity for protein synthesis. You can think of the genetic information in an mRNA molecule as a series of sequential, non-overlapping three-letter "words." Each sequence of three nucleotides (the three "letters") along the chain specifies a particular amino acid. Each three-letter "word" is called a codon. Each codon is complementary to the corresponding triplet in the DNA molecule from which it was transcribed.

The complete genetic code is shown in Figure 12.5. Notice that there are many more codons than there are different amino acids in proteins. Combinations of the four available "letters" (the bases) give 64 (4^3) different three-letter codons, yet these codons determine only 20 amino acids. AUG, which codes for methionine, is also the start codon, the initiation signal for translation. Three of the codons (UAA, UAG, UGA) are stop codons, or chain terminators; when the translation machinery reaches one of these codons, translation stops, and the polypeptide is released from the translation complex.

After describing the properties of the genetic code, we will examine some of the scientific thinking and experimentation that went into deciphering it.

224 CHAPTER TWELVE

Second letter



72.5 The Universal Genetic Code

Genetic information is encoded in mRNA in three-letter units—codons—made up of the bases uracil (U), cytosine (C), adenine (A), and guanine (G). To decode a codon, find its first letter in the left column, then read across the top to its second letter, then read down the right column to its third letter. The amino acid the codon specifies is given in the corresponding row. For example, AUG codes for methionine, and GUA codes for valine.

The genetic code is redundant but not ambiguous

After the start and stop codons, the remaining 60 codons are far more than enough to code for the other 19 amino acids—and indeed there are repeats. Thus we say that the genetic code is redundant; that is, an amino acid may be represented by more than one codon. The redundancy is not evenly divided among the amino acids. For example, methionine and tryptophan are represented by only one codon each, whereas leucine is represented by six different codons (see Figure 12.5).

The term redundancy should not be confused with ambiguity. To say that the code was ambiguous would mean that a single codon could specify either of two (or more) different amino acids; there would then be doubt whether to put in, say, leucine or something else. The genetic code is not ambiguous. Redundancy in the code means that there is more than one clear way to say, "Put leucine here." In other words, a given amino acid may be encoded by more than one codon, but a codon can code for only one amino acid. But just as people in different places prefer different ways of saying the same thing—"Good-bye!" "See you!" "Ciao!" and "So long!" have the same meaning—different organisms prefer one or another of the redundant codons.

The genetic code appears to be nearly universal, applying to all the species on our planet. Thus the code must be an ancient one that has been maintained intact throughout the evolution of living things. Exceptions are known: Within mitochondria and chloroplasts, the code differs slightly from that in prokaryotes and in the nuclei of eukaryotic cells; in one group of protists, UAA and UAG code for glutamine rather than functioning as stop codons. The significance of these differences is not yet clear. What is clear is that the exceptions are few and slight.

The common genetic code means that there is also a common language for evolution. As natural selection has

resulted in one species replacing another, the raw material of genetic variation has remained the same: DNA sequences with the same "meaning." The common code also has great implications in genetic engineering, as we will see in Chapter 17, since it means that a human gene is in the same language as a bacterial gene. So the protein transcription and translation machinery of a bacterium can utilize genes from a human as well as its own genes.

The codons in Figure 12.5 are mRNA codons. The master codons on the DNA strand that was transcribed to produce the mRNA are complementary and antiparallel to these codons. Thus, for example, 3'-AAA-5' in the template DNA strand corresponds to phenylalanine (which is coded for by the mRNA codon 5'-UUU-3'), and 3'-ACC-5' in the template DNA corresponds to tryptophan (which is coded for by the mRNA codon 5'-UGG-3').

Biologists broke the genetic code by using artificial messengers

Molecular biologists broke the genetic code in the early 1960s. The problem seemed difficult: How could more than 20 "code words" be written with an "alphabet" consisting of only four "letters"? How, in other words, could four bases code for 20 or so different amino acids?

That the code was a triplet code, based on three-letter codons, was considered likely. Since there are only four letters (A, G,

C, U), a one-letter code clearly could not unambiguously encode 20 amino acids; it could encode only four of them. A two-letter code could contain only $4 \times 4 = 16$ codons—still not enough. But a triplet code could contain up to $4 \times 4 \times 4 = 64$ codons.

Marshall W. Nirenberg and J. H. Matthaei, at the National Institutes of Health, made the first decoding breakthrough in 1961 when they realized that they could use a very simple artificial polynucleotide instead of a complex natural mRNA as a messenger. They could then identify the polypeptide that the artificial messenger encoded.

Nirenberg prepared an artificial mRNA in which all the bases were uracil (poly U). When poly U was added to a test tube containing all the ingredients necessary for protein synthesis (ribosomes, amino acids, activating enzymes,

FROM DNA TO PROTEIN: GENOTYPE TO PHENOTYPE 225

72.6 Deciphering the Genetic Code

Nirenberg and Matthaei used a test-tube protein synthesis system to determine the amino acids specified by synthetic mRNA's of known codon composition.

EXPERIMENT

Question: What are the amino acids specified by the triplet codons UUU, AAA, and CCC?

METHOD

Q Prepare a bacterial extract containing all the components needed to make proteins except mRNA.

Q Add an artificial mRNA containing only one repeating base.

RESULTS

§} Depending on the base in the mRNA, the polypeptide produced contains a single amino acid.

I

tRNA's, and other factors), a polypeptide formed. This polypeptide contained only one kind of amino acid: phenylalanine (Phe). Poly U coded for poly Phe! Accordingly, UUU appeared to be the mRNA code word—the codon—for phenylalanine. Following up on this success, Nirenberg and Matthaei soon showed that CCC codes for proline and AAA for lysine (Figure 12.6). (Poly G presented some chemical problems and was not tested initially.) UUU, CCC, and AAA were three of the easiest codons; different approaches were required to work out the rest.

Other scientists later found that simple artificial mRNA's only three nucleotides long—each amounting to a codon— could bind to a ribosome, and that the resulting complex could then cause the binding of the corresponding tRNA covalently bound to its specific amino acid. Thus, for example, simple UUU causes the tRNA charged with phenylalanine to bind to the ribosome. After this discovery, complete deciphering of the genetic code was relatively simple. To find the "translation" of a codon, Nirenberg could use a sample of that codon as an artificial mRNA and see which amino acid became bound to it.

Preparation for Translation: Linking RNA's, Amino Acids, and Ribosomes

How is the information contained in mRNA translated into proteins? Recall that Crick proposed that there is an adapter, something that can recognize the information in an mRNA codon and also bind the amino acid specified by the codon. That adapter is tRNA.

Translation occurs at ribosomes, which are molecular protein-synthesizing machines that hold the mRNA and tRNA's in place. In prokaryotes, ribosomes bind to mRNA as it is made, coupling the processes of transcription and translation. In eukaryotes, mRNA leaves the nucleus and is bound to ribosomes in the cytoplasm.

Two key events must take place to ensure that the protein made is the one specified by mRNA:

► tRNA must read mRNA correctly.

► tRNA must carry the amino acid that is correct for its reading of the mRNA.

At first glance, these two events seem similar, but they are quite distinct, as you will see.

^^T^^^r^^^Ta

Phe!Phe; Phe

Lys I Lys . Lys

Pro I Pro I Pro

Conclusion: UUU is an mRNA codon for phenylalanine. AAA is an mRNA codon for lysine. CCC is an mRNA codon for proline.

Transfer RNA's carry specific amino acids and bind to specific codons

The codon in mRNA and the amino acid in a protein are related by way of an adapter—a specific tRNA. For each of the 20 amino acids, there is at least one specific type of tRNA molecule.

The structure of the tRNA molecule relates clearly to its functions: It carries an amino acid, it associates with mRNA molecules, and it interacts with ribosomes. A tRNA molecule has about 75 to 80 nucleotides. It has a three-dimensional shape that is maintained by complementary base pairing (hydrogen bonding) within its own sequence (Figure 12.7).

At the 3' end of every tRNA molecule is a site to which its specific amino acid binds covalently. At about the midpoint is a group of three bases, called the anticodon, that constitutes the point of contact with mRNA. Each tRNA species has a unique anticodon, which is complementary to the mRNA codon for that tRNA's amino acid. The codon and the anticodon unite by complementary base pairing (hydrogen bonding). At contact, they are antiparallel to each other.

As an example of this process, consider the amino acid arginine:

- ▶ The DNA coding region for arginine is 3'-GCC-5', which is transcribed, by base pairing, to...
- ▶ the mRNA codon 5'-CGG-3', which binds to...
- ▶ a tRNA with the anticodon 3'-GCC-5'.

Recall that 61 different codons encode the 20 amino acids in proteins (see Figure 12.5). Does this mean that the cell must produce 61 different tRNA species, each with a different anticodon? No. The cell gets by with about two-thirds that number of tRNA species, because the specificity for the

226 CHAPTER TWELVE

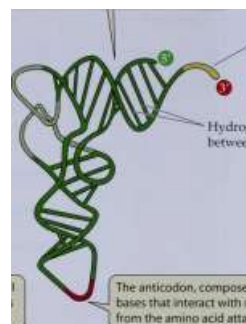
| This computer-generated, space-filling representation shows the three-dimensional structure of a tRNA.



I This three-dimensional representation emphasizes the internal regions of base pairing.

I This flattened "cloverleaf" model emphasizes base pairing between complementary nucleotides.

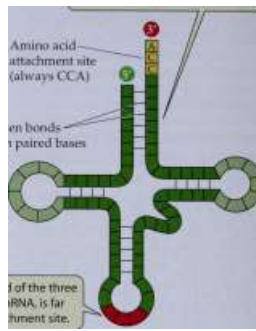
This icon for tRNA will be used in the figures that follow.



Amino acid attachment site (always CCA)

Hydrogen bonds between paired bases

The anticodon, composed of the three bases that interact with mRNA, is far from the amino acid attachment site.



72.7 Transfer RNA: Crick's Adapter

The tRNA molecule carries amino acids, associates with mRNA molecules, and interacts with ribosomes. There is at least one specific tRNA molecule for each of the amino acids.

base at the 3' end of the codon (and the 5' end of the anticodon) is not always strictly observed. This phenomenon, called wobble, allows the alanine codons GCA, GCC, and GCU all to be recognized by the same tRNA. Wobble is allowed in some matches but not in others; of most importance, it does not allow the genetic code to be ambiguous!

The three-dimensional shape of tRNAs (see Figure 12.7) allows them to combine specifically with binding sites on ribosomes. The structure of tRNA molecules relates clearly to their functions: They carry amino acids, associate with mRNA molecules, and interact with ribosomes.

Activating enzymes link the right tRNA's and amino acids

How does a tRNA molecule combine with the correct amino acid for the codon it recognizes? A family of activating enzymes, known more formally as aminoacyl-tRNA synthetases, accomplishes this task (Figure 12.8). Each activating enzyme is specific for one amino acid and for its tRNA. The enzyme has a three-part active site that recognizes three smaller molecules: a specific amino acid, ATP, and a specific tRNA.

The enzyme reacts with tRNA and an amino acid in two steps:

enzyme + ATP + AA \rightarrow enzyme-AMP-AA + PP ;

enzyme-AMP-

-AA + tRNA \rightarrow enzyme + AMP + tRNA-AA

The amino acid is attached to the 3' end (a free OH group on the ribose) of tRNA with a high-energy bond, forming charged tRNA. This bond will provide the energy for the synthesis of the peptide bond that will join adjacent amino acids.

A clever experiment by Seymour Benzer and his colleagues at the California Institute of Technology showed the importance of the specificity of the attachment of tRNA to its amino acid. The amino acid cysteine, already properly attached to its tRNA, was chemically modified to become a different amino acid, alanine. Which component—the amino acid or the tRNA—would be recognized when this hybrid charged tRNA was put into a protein-synthesizing system? The answer was: the tRNA. Everywhere in the synthesized protein where cysteine was supposed to be, alanine appeared instead. The cysteine-specific tRNA delivered its cargo (alanine) to every address where cysteine was called for. This experiment showed that the protein synthesis machinery recognizes the tRNA part of charged tRNA, not the amino acid part.

If activating enzymes in nature did what Benzer did in the laboratory and put the wrong amino acids on tRNA's, those amino acids would be inserted into proteins at inappropriate places, leading to alterations in protein shape and function. The fact that the activating enzymes are highly specific has led to the process of linking tRNA and amino acid being called the "second genetic code."

The ribosome is the staging area for translation

Ribosomes are required for the translation of the genetic information in mRNA into a polypeptide chain. Although ribosomes are the smallest cellular organelles, their mass of several million daltons makes them large in comparison with charged tRNA's.

Each ribosome consists of two subunits, a large one and a small one (Figure 12.9). In eukaryotes, the large subunit consists of three different molecules of rRNA and about 45 different protein molecules, arranged in a precise pattern.

£i/l!ifr >

I The activating enzyme, an aminoacyl-tRNA synthetase, has a three-part active site that recognizes three smaller molecules. A specific synthetase is required for each amino acid.

I The enzyme activates the amino acid, catalyzing a reaction with ATP in which high energy AMP-amino acid and a pyrophosphate ion form.



Specific amino acid •(AA)

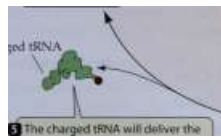


Amino acid site ATP site

tRNA site

The enzyme may begin again.

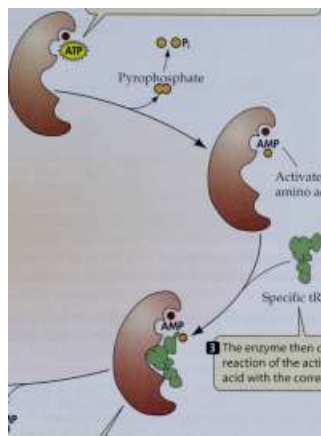
Charged tRNA



| The charged tRNA will deliver the appropriate amino acid to join the elongating polypeptide product of translation.



tRNA-AA



Activated amino acid

72.8 Charging a tRNA Molecule

Each activating enzyme must make the correct association of an amino acid and its tRNA. The enzyme is an essential link between nucleic acid "language" and protein "language."

T

AMP O

Specific tRNA

§ The enzyme then catalyzes a reaction of the activated amino acid with the correct tRNA.

Q The specificity of the enzyme ensures that the correct amino acid and tRNA have been brought together.

The small subunit consists of one rRNA molecule and 33 different protein molecules. When not active in the translation of mRNA, the ribosomes exist as separated subunits.

The ribosomes of prokaryotes are somewhat smaller than those of eukaryotes, and their ribosomal proteins and RNA's are different. Mitochondria and chloroplasts also contain ribosomes, some of which are even smaller than those of prokaryotes.

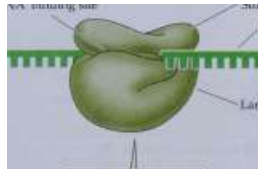
The different proteins and RNA's in a subunit are held together by ionic and hydrophobic forces, not covalent bonds. If these forces are disrupted by detergents, for example, the proteins and rRNA's separate from each other. When the detergent is removed, the entire complex structure self-assembles. This is like separating the pieces of a jigsaw puzzle and having them fit together again without human hands to guide them!

mRNA binding site

A given ribosome is not specifically adapted to produce just one kind of protein. A ribosome can combine with any mRNA and all tRNA's, and thus can be used to make many different polypeptide products. The mRNA contains the information that specifies the polypeptide sequence; the ribosome is simply the molecular factory where the task is accomplished. Its structure enables it to hold the mRNA and tRNA's in the right positions, thus allowing the growing polypeptide to be assembled efficiently.

12.9 Ribosome Structure

Each ribosome consists of a large and a small subunit, which separate when they are not in use for protein synthesis.



Small subunit mRNA

Large subunit



Ribosomes are irregularly shaped and composed of two subunits.

There are 4 sites for tRNA binding. Codon-anticodon interactions between tRNA and mRNA only occur at the P and A sites.

228 CHAPTER TWELVE

Initiation



mRNA

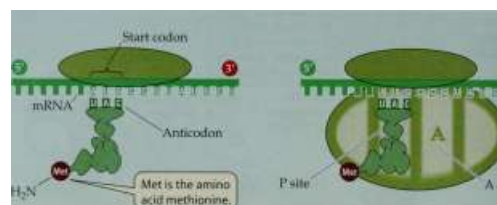
Ribosome

recognition

sequence

f

The small ribosomal subunit binds to its recognition sequence on mRNA.



A site

I Methionine-charged tRNA binds the AUG initiation codon, completing the initiation complex.

12.10 The Initiation of Translation

Translation begins with the formation of an initiation complex.

| The large ribosomal subunit joins the initiation complex, with methionine-charged tRNA now occupying the P site.

On the large subunit of the ribosome there are four sites to which tRNA binds (see Figure 12.9). A tRNA traverses these four sites in order:

- The T (transfer) site is where a tRNA carrying an amino acid first lands on the ribosome, accompanied by a special protein "escort" called the T, or transfer, factor.
- The A (amino acid) site is where the tRNA anticodon binds to mRNA codon, thus lining up the correct amino acid to be added to the growing polypeptide chain.
- The P (polypeptide) site is where the tRNA adds its amino acid to the growing polypeptide chain.
- The E (exit) site is where the tRNA, having given up its amino acid, resides before leaving the ribosome and going back to the cytosol to pick up another amino acid and begin the process again.

Because codon-anticodon interactions and peptide bond formation occur at the A and P sites, we will describe their function in detail in the next section.

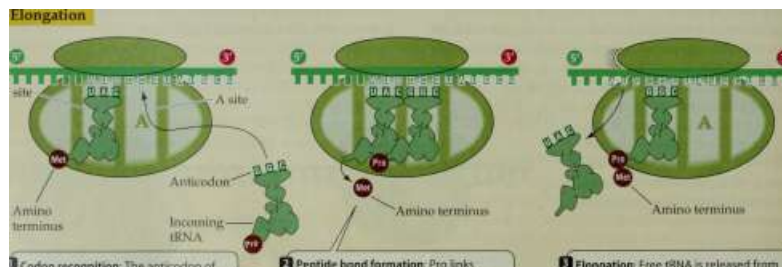
Translation: RNA-Directed Polypeptide Synthesis

We have been working our way through the steps by which the sequence of bases in the template strand of a DNA molecule specifies the sequence of amino acids in a protein (see Figure 12.3). We are now at the last step: translation, the RNA-directed assembly of a protein. Like transcription, translation occurs in three steps: initiation, elongation, and termination.

Translation begins with an initiation complex

The translation of mRNA begins with the formation of an initiation complex, which consists of a charged tRNA bearing what will be the first amino acid of the polypeptide chain and a small ribosomal subunit, both bound to the mRNA (Figure 12.10). The small ribosomal subunit binds to a sequence that it recognizes on the mRNA. This sequence

Psite



I Codon recognition: The anticodon of tRNA binds to the codon exposed at the A site.

f

Peptide bond formation: Pro links to Met.

I Elongation: Free tRNA is released from the P site, and the ribosome shifts by one codon, so the growing polypeptide moves to the P site. The free tRNA is released via the E site.

is "upstream" (toward the 5' end) of the actual start codon that begins translation.

Recall that the start codon in the genetic code is AUG (see Figure 12.5). Thus the first amino acid in the chain is always methionine. The anticodon of a methionine-charged tRNA binds to the mRNA start codon by complementary base pairing. (Not all mature proteins have methionine as their N-terminal amino acid, however. In many cases, the initiator methionine is later removed by an enzyme.)

After the methionine-charged tRNA has bound to the mRNA, the large subunit of the ribosome joins the complex. The charged tRNA, bearing methionine, now lies in the P site of the ribosome, and the A site is aligned with the second codon.

How are all these ingredients—mRNA, two ribosomal subunits, and methionine-charged tRNA—put together properly? A group of proteins called initiation factors help direct the process, using GTP as an energy supply.

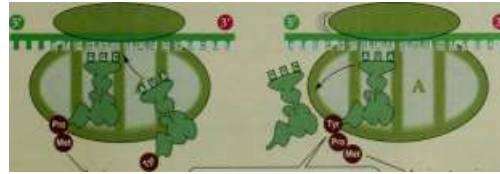
The polypeptide elongates from the N terminus

During translation, the ribosome moves along the mRNA in the 5'-to-3' direction (Figure 12.11). A charged tRNA whose anticodon is complementary to the second codon on the mRNA enters the open A site of the large ribosomal subunit. The large subunit then catalyzes two reactions, collectively called peptidyl transferase activity:

- breakage of the bond between the tRNA in the P site and its amino acid
- peptide bond formation between this amino acid and the one attached to the tRNA in the A site

In this way, methionine (the amino acid in the P site) becomes the N terminus of the new protein. The second amino acid is now bound to methionine, but remains attached to its tRNA by its carboxyl group (—COOH) in the A site.

What catalyzes this binding? In 1992, Harry Noller and his colleagues at the University of California at Santa Cruz



Amino terminus

t

Peptide bond formation: Tyr links to the growing polypeptide.

found that if they removed almost all the proteins in the large ribosomal subunit, it still catalyzed peptide bond formation. But if the rRNA was destroyed, so was peptidyl transferase activity. Part of the rRNA in the large subunit interacts with the end of the charged tRNA where the amino acid is attached. Thus rRNA appears to be the catalyst.

The idea that RNA—instead of the usual enzymes—can act as a catalyst, or ribozyme, was surprising, but is not so far-fetched. Because of its base-pairing ability, RNA can fold into three-dimensional shapes (see Figure 12.7) and bind substrates, just as protein-based enzymes do.

Elongation continues and the polypeptide grows

After the first tRNA releases its methionine, it dissociates from the ribosome, returning to the cytosol to become charged with another methionine. The second tRNA, now bearing a dipeptide, shifts to the P site of the ribosome, which moves along the mRNA by another codon. Energy for this movement comes from the hydrolysis of another molecule of GTP.

The elongation process continues, and the polypeptide chain grows, as the steps are repeated:

- ▶ The next charged tRNA enters the open A site.
- ▶ Its amino acid forms a peptide bond with the amino acid chain in the P site, so that it picks up the growing polypeptide chain from the tRNA in the P site.
- ▶ The tRNA in the P site is released. The ribosome shifts one codon, so that the entire tRNA- polypeptide complex, along with its codon, moves to the newly vacated P site.

All these steps are assisted by proteins called elongation factors.

A release factor terminates translation

How does the elongation cycle end? When a stop codon— UAA, UAG, or UGA—enters the A site, translation terminates (Figure 12.12). These codons encode no amino acids, nor do they bind any tRNA. Rather, they bind a protein release factor, which causes a water molecule instead of an amino acid to attach to the forming protein.

The newly completed protein thereupon separates from the ribosome. Its C terminus is the last amino acid to join the chain. Its N terminus, at least initially, is methionine, as a consequence of the AUG start codon. In its amino acid sequence, it contains information for its three-dimensional shape, as well as its ultimate cellular destination.

'Amino terminus

Q Codon recognition: An anticodon of tRNA bearing tyrosine (Tyr) binds to the codon at the A site.

Q Elongation: Free tRNA is released from the P site, and the ribosome advances, so the growing polypeptide moves to the P site.



[ril^ 12.11 Translation: The Elongation Stage

The polypeptide chain elongates as the mRNA is translated.

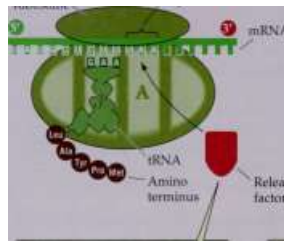
230 CHAPTER TWELVE

Termination

Ribosome

Stop codon

Small subunit



t Release factor binds to the complex when a stop codon is in the A site.

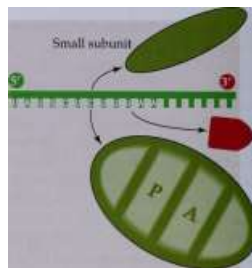
Release factor



COO

Polypeptide

J



Q Releasing the polypeptide product: The release factor disconnects the polypeptide from the tRNA in the P site, freeing both the polypeptide and the tRNA.

Large subunit

ijThe remaining components (mRNA, small ribosomal subunit, and large ribosomal subunit) separate.

12.12 The Termination of Translation

Translation terminates when the ribosome encounters a stop codon on the mRNA.

Table 12.1 summarizes the initiation and termination of transcription and translation.

Regulation of Translation

Like any factory, the machinery of translation can work at varying rates. For example, externally applied chemicals, such as some antibiotics, can stop translation. Conversely, the presence of more than one ribosome on an mRNA can speed up protein synthesis.

Some antibiotics work by inhibiting translation

Antibiotics are defense molecules produced by microorganisms such as certain bacteria and fungi. These substances often destroy other microbes, which might compete with the defender for nutrients. Since the 1940s, scientists have isolated increasing numbers of antibiotics, and physicians use them to treat a great variety of infectious diseases, ranging from bacterial meningitis to pneumonia to gonorrhea.

The key to the medical use of antibiotics is specificity: An antibiotic must work to destroy the microbial invader, but not harm the human host. One way in which antibac-

12.1

Signals that Start and Stop Transcription and Translation

TRANSCRIPTION

TRANSLATION

Initiation Termination

Promoter sequence in DNA

Terminator sequence in DNA

AUG start codon

UAA, UAG, or UGA stop codon in mRNA

terial antibiotics accomplish this task is to block the synthesis of the bacterial cell wall, something that is essential to the microbe but that is not part of human biochemistry. Penicillin works in this fashion.

Another way in which antibiotics work is to inhibit bacterial protein synthesis. Recall that the bacterial ribosome is smaller, and has a different collection of proteins, than the eukaryotic ribosome. Some antibiotics bind only to bacterial ribosomal proteins that are important in protein synthesis (Table 12.2). Without the ability to make proteins, the bacterial invaders die, and the infection is stemmed.

Polysome formation increases the rate of protein synthesis

Several ribosomes can work simultaneously at translating a single mRNA molecule, producing multiple molecules of the protein at the same time. As soon as the first ribosome has moved far enough from the initiation point, a second initiation complex can form, then a third, and so on. An assemblage consisting of a thread of mRNA with its beadlike ribosomes and their growing polypeptide chains is called a polyribosome, or polysome (Figure 12.13). Cells that are actively synthesizing proteins contain large numbers of polysomes and few free ribosomes or ribosomal subunits.

12.2

Antibiotics that Inhibit Bacterial Protein Synthesis

ANTIBIOTIC

STEP INHIBITED

Chloromycetin Erythromycin

Neomycin

Streptomycin Tetracycline

Formation of peptide bonds Translocation of mRNA along

ribosome Interactions between tRNA and

mRNA Initiation of translation Binding of tRNA to ribosome

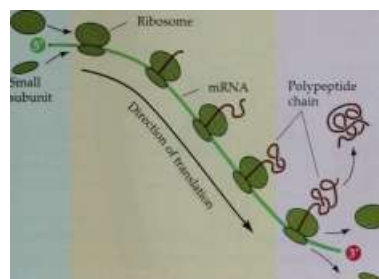
(a)

Large subunit

Small subunit

Ribosome

Polypeptide chain



72.73 A Polysome

(a) A polysome consists of ribosomes and their growing polypeptide chains moving in single file along an mRNA molecule, (b) An electron microscopic view of a polysome.

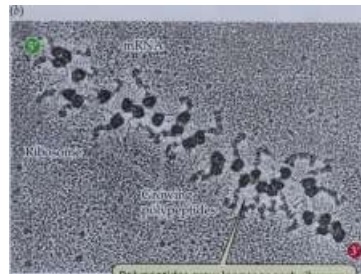
A polysome is like a cafeteria line, in which patrons follow each other, adding items to their trays. At any moment, the person at the start has a little food (a newly initiated protein); the person at the end has a complete meal (a completed protein). However, in the polysome cafeteria, everyone gets the same meal: Many copies of the same protein are made from a single mRNA.

Posttranslational Events

The functional protein that results from protein synthesis is not necessarily the same as the polypeptide chain that is released from the ribosome. Especially in eukaryotic cells, the polypeptide may need to be moved far from the site of synthesis in the cytoplasm, to an organelle, or even secreted from the cell. In addition, the polypeptide is often modified by the addition of new chemical groups that have functional significance. In this section, we examine these two posttranslational aspects of protein synthesis.

Chemical signals in proteins direct them to their cellular destinations

As a polypeptide chain forms on the ribosome, it spontaneously folds into its three-dimensional shape. As described in Chapter 3, this shape is determined by the particular sequence of the amino acids that make up the protein, as well as factors such as the polarity and charge of their R groups. Ultimately, this shape allows the polypeptide to interact with other molecules in the cell, such as a substrate or another polypeptide. In addition to this structural information, the amino acid sequence contains an "address label" indicating where in the cell the polypeptide belongs.



Polypeptides grow longer as each ribosome moves toward the 3' end of mRNA.

All protein synthesis begins on free ribosomes in the cytoplasm. As a polypeptide chain is made, the information contained in its amino acid sequence gives it one of two sets of instructions (Figure 12.14):

- Finish translation and be released to the cytoplasm. Such proteins are sent to the nucleus, mitochondria, plastids, or peroxisomes, depending on the address in their instructions; or, lacking such specific instructions, they remain in the cytoplasm.
- Stop translation, go to the endoplasmic reticulum (ER), and finish synthesis there. Such proteins are sent to the lysosomes (via the Golgi apparatus) or the plasma membrane, are instructed to remain in the ER; or, lacking such specific instructions, they are secreted from the cell.

destination: cytoplasm. After translation, some folded polypeptides have a short exposed sequence of amino acids that acts like a postal "zip code," directing them to an organelle. These signal sequences are either at the N terminus or in the interior of the amino acid chain. For example, the following sequence is a nuclear localization signal:

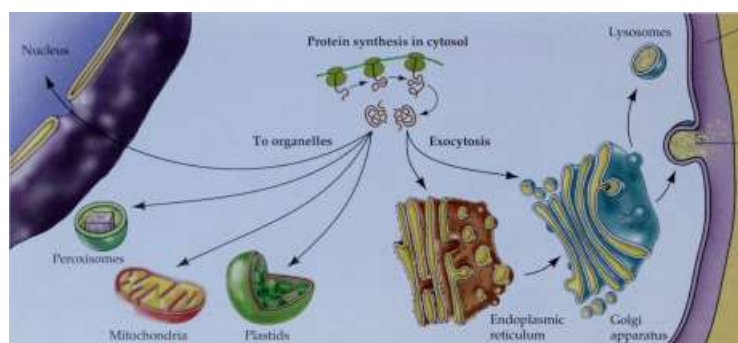
—Pro—Pro—Lys—Lys—Lys—Arg—Lys—Val—

This amino acid sequence occurs, for example, in histone proteins, but not in citric acid cycle enzymes, which are addressed to the mitochondria.

The signal sequences have a conformation that allows them to bind to specific receptor proteins, appropriately called docking proteins, on the outer membrane of the appropriate organelle. Once the protein has bound to it, the receptor forms a channel in the membrane, allowing the protein to pass through to its organelle destination (it is usually unfolded by a chaperonin so that it can pass through the channel).

destination: endoplasmic reticulum. If a specific hydrophobic sequence of about 25 amino acids occurs at the beginning of a polypeptide chain, the finished product is

232 CHAPTER TWELVE



Plasma membrane

Exocytosis

Extracellular medium

Mitochondria

Plastids

12.14 Destinations for Newly Translated Proteins in a Eukaryotic Cell

Signal sequences on newly synthesized polypeptides bind to specific receptor proteins on the outer membranes of the organelle to which they are "addressed." Once the protein has bound to it, the receptor forms a channel in the membrane and the protein enters the organelle.

[Protein synthesis begins on ribosomes not attached to endoplasmic reticulum.

...on which the signal sequence of amino acids is present.

I The signal recognition particle binds to a receptor protein in the membrane of the ER.

| The signal recognition particle is released. The signal sequence passes through a channel in the membrane.

Interior of rough endoplasmic reticulum

Signal

recognition Receptor protein

particle __, '

-flB-

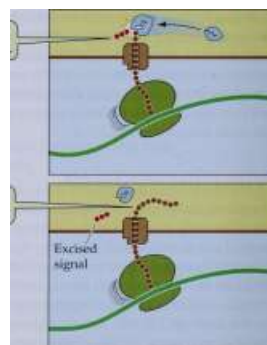
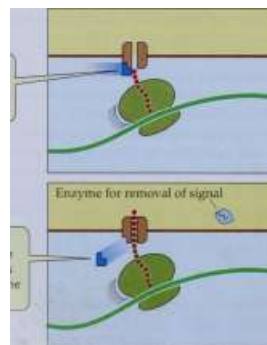
^Ribosome Cytosol of cell

tThe signal sequence is 1 removed inside the ER.



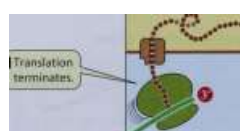
f

The polypeptide continues to elongate. J



Q Translation

I terminates.



Completed /"" ^ protein

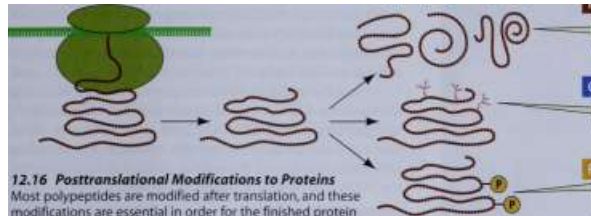
I ^^v Ribosome g* /released

72.75 A Signal Sequence Moves a Polypeptide into the ER

When a signal sequence of amino acids is present at the beginning of the polypeptide chain, the polypeptide will be taken into the endoplasmic reticulum. The finished protein is thus segregated from the cytosol.

Translation

Posttranslational processing



Proteolysis

J Cleaving the polypeptide allows the fragments to fold into different shapes.

Glycosylation

Adding sugars is important for targeting and recognition.

72.76 Posttranslational Modifications to Proteins

Most polypeptides are modified after translation, and these modifications are essential in order for the finished protein to function properly.

destined for the ER, lysosomes, and plasma membrane, or for secretion. The signal sequence attaches to a signal recognition particle composed of protein and RNA (Figure 12.15). This attachment blocks further protein synthesis until the ribosome can become attached to a specific receptor protein in the membrane of the ER. Once again, the receptor protein becomes a channel through which the growing polypeptide passes, either into the ER membrane itself or into the interior of the ER. An enzyme within the ER interior then removes the signal sequence from the polypeptide chain. At this point, protein synthesis resumes, and the chain grows longer. From the ER, the newly formed protein can be transported to its appropriate location—to other cellular compartments or to the outside of the cell—without mixing with other molecules in the cytoplasm.

Additional signals are needed for sorting the protein further (remember that its ER signal sequence has been removed). These signals are of two kinds. Some are sequences of amino acids that allow the protein's retention within the ER. Others are sugars added in the Golgi apparatus, to which the protein is transferred from the ER; the resulting glycoproteins end up either at the plasma membrane or in a lysosome (or plant vacuole), depending on which sugars are added. Proteins with no signals pass from the ER through the Golgi apparatus and are secreted from the cell.

It is important to emphasize that the addressing of a protein to its destination is a property of its amino acid sequence, and so is genetically determined. An example of what can go wrong if a gene for protein targeting is mutated is mucopolidosis II, or I-cell disease. People with this disease lack an essential enzyme for the formation of the lysosomal targeting signal. So proteins destined for their lysosomes never get there, and instead either stay in the Golgi (where they form I, or inclusion, bodies) or are secreted from the cell. The inability to perform normal lysosome functions leads to progressive illness and death in childhood.

Many proteins are modified after translation

It is the exception and not the rule that the finished protein product is identical to the polypeptide chain translated from mRNA on the ribosomes. Instead, most polypeptides are modified after translation, both covalently and nonco-

Phosphorylation

Added phosphate groups alter the shape of the protein.

valently. In both cases, the modifications are essential to the final functioning of the protein. Some kinds of covalent changes include the following (Figure 12.16):

► Proteolysis is the cutting of a polypeptide chain. Cleavage of the signal sequence from the growing polypeptide chain in the ER is an example of proteolysis; the protein might go back out of the ER through the membrane channel if the signal sequence were not cut off. Some proteins are actually made from polypeptides (long polypeptides) that are cut into final products by enzymes called proteases. Viruses such as HIV encode such proteases, which are essential because the large viral polypeptide cannot fold properly unless it is cut. Certain drugs used to treat AIDS work by inhibiting HIV's protease.

► Glycosylation involves the addition of sugars to proteins. In both the ER and the Golgi, resident enzymes catalyze the

addition of various sugar residues or short sugar chains to certain amino acid R groups on proteins as they pass through. One type of "sugar coating" is essential for addressing proteins to lysosomes, as we saw above. Other types are important in the three-dimensional structure and recognition of proteins at the cell surface. Still others help in stabilizing proteins stored in storage vacuoles in plant seeds.

► Phosphorylation is the addition of phosphate groups to proteins and is catalyzed by protein kinases. These charged groups change the three-dimensional structures of targeted proteins, often exposing an active site of an enzyme, or a binding site for another protein—as we will see in Chapter 15.

Mutations: Heritable Changes in Genes

Accurate DNA replication, transcription, and translation all depend on the reliable pairing of complementary bases. Errors occur, though infrequently, in all three processes. Errors happen least often in DNA replication. However, the consequences of those errors can be most severe because only they are heritable.

Mutations are heritable changes in genetic information. In unicellular organisms, any mutations that occur are passed on to the daughter cells when the cell divides. In multicellular organisms, there are two general types of mutations in terms of inheritance:

234 CHAPTER TWELVE

► Somatic mutations are those that occur in non-gamete body cells. These mutations are passed on to the daughter cells after mitosis, and to the offspring of these cells in turn. A mutation in a single skin cell, for example, could result in a patch of skin cells, all with the same DNA alteration.

► Germ-line mutations are those that occur in the cells that give rise to gametes. A gamete with the mutation passes it on to a new organism at fertilization.

Very small changes in the genetic material often lead to easily observable changes in the phenotype. Some effects of mutations in humans are readily detectable—dwarfism, for instance, or the presence of more than five fingers on each hand. A mutant genotype in a microorganism may be obvious if, for example, it results in a change in nutritional requirements, as we described for *Neurospora* earlier (see Figure 12.1).

Other mutations may be unobservable. In humans, for example, a particular mutation drastically lowers the level of an enzyme called glucose 6-phosphate dehydrogenase that is present in many tissues, including red blood cells. The red blood cells of a person carrying the mutant allele are abnormally sensitive to an antimalarial drug called primaquine; when such people are treated with this drug, their red blood cells rupture, causing serious medical problems. People with the normal allele have no such problem. Before the drug came into use, no one was aware that such a mutation existed. Similarly, distinguishing a mutant bacterium from a normal bacterium may require sophisticated chemical methods, not just visual inspection.

Some mutations cause their phenotypes only under certain restrictive conditions and are not detectable under other, permissive conditions. We call organisms carrying such mutations conditional mutants. Many conditional mutants are temperature-sensitive, able to grow normally at a permissive temperature—say, 30°C—but unable to grow at a higher restrictive temperature—say, 37°C. The mutant allele in such an organism may code for an enzyme with an unstable tertiary structure that is altered at the restrictive temperature.

All mutations are alterations in the nucleotide sequence of DNA. We divide mutations into two categories:

► Point mutations are mutations of single genes: One allele becomes another because of small alterations in the sequence or number of nucleotides—even as small as the substitution of one nucleotide for another.

► Chromosomal mutations are more extensive alterations. They may change the position or direction of a DNA segment without actually removing any genetic information, or they may cause a segment of DNA to be irretrievably lost.

Point mutations are changes in single bases

Point mutations result from the addition or subtraction of a nucleotide base, or the substitution of one base for another, in the DNA, and hence in the mRNA. Point mutations can be caused by errors in chromosome replication that are not corrected in proofreading, or by environmental mutagens such as chemicals and radiation.

Because of the redundancy of the genetic code, some point mutations result in no change in amino acids when the altered mRNA is translated; for this reason they are called silent or synonymous mutations. For example, four mRNA codons code for proline: CCA, CCC, CCU, and CCG (see Figure 12.5). If the template strand of DNA has the sequence CGG, it will be transcribed to CCG in mRNA, and proline-charged tRNA will bind at the ribosome. But if there is a mutation such that the codon in the template DNA now reads AGG, the mRNA codon will be CCU—and the tRNA that binds will still carry proline:

Synonymous mutation

Mutation at position 12 in DNA: C→A

DNA

template

strand

Q

CGAGGGGCTAATT

[©

I

Transcription

mRNA

Peptide

©:

\

Translation

MetT Trp T Leu T Pro TAsp Stop

Result: No change in amino acid sequence

Silent mutations are quite common, and account for genetic diversity that is not expressed as phenotypic differences.

In contrast to silent mutations, some base substitution mutations may change the genetic message such that one amino acid substitutes for another in the protein. These are missense mutations:

Missense mutation

Mutation at position 14 in DNA: T → A

DNA

template

strand

©

CACCGAGGGCC AATT

[©

I

Transcription

mRNA

©"

:©

I

Translation

Met! Trp I LeuT Pro TVal Stop

Peptide

Result: Amino acid change at position 5: Asp → Val

A specific example of a missense mutation is the sickle-cell allele for human (3-globin. Sickle-cell disease results from a defect in hemoglobin, a protein that carries oxygen. The gene for fi-globin, one of the polypeptides in hemoglobin, differs by one amino acid between the normal and the sickle-cell allele. Persons who are homozygous for this recessive allele have defective red blood cells. Where oxygen is abundant, as in the lungs, the cells are normal in structure and function. But at the low oxygen levels characteristic of working muscles, the red blood cells collapse into the shape of a sickle (Figure 12.17).

A missense mutation may sometimes cause a protein not to function, but often the effect is only to reduce the functional efficiency of the protein. Therefore, individuals carrying missense mutations may survive, even though the affected protein is essential to life. Through evolution, some missense mutations even improve functional efficiency.

Nonsense mutations, another type of mutation in which bases are substituted, are more often disruptive than are missense mutations. In a nonsense mutation, the base substitution causes a chain terminator (stop) codon, such as UAG, to form in the mRNA product:

Nonsense mutation

Mutation at position 5 in DNA: C → T

DNA

template

strand

TACACGAGGGCCTAATT

©

\

Transcription

mRNA

©IB

AUGU GCUCCCGGAUUA.

I Translation

Peptide OZ

Result: Only one amino acid translated; no protein made

The result is a shortened protein product, since translation does not proceed beyond the point where the mutation occurred.

Not all point mutations are base substitutions. Single base pairs may be inserted into or deleted from DNA. Such mutations are known as frame-shift mutations because they interfere with the decoding of the genetic message by throwing it out of register:

Frame-shift mutation

Mutation by insertion of T between bases 6 and 7 in DNA

DNA

template

strand

DNA

template

strand

•TACACCGAGGGCCTAATT

B-L

mRNA

Peptide

©:

•TACACCGAGGGCCTAATT

I Transcription TTTT'H't frClCCCCG&AUtiAX

L©

L©

©

Translation

Met TrpIThrIProIGIylLeu

Result: All amino acids changed beyond the insertion

Think again of codons as three-letter words, each corresponding to a particular amino acid. Translation proceeds codon by codon; if a base is added to the message or subtracted from it, translation proceeds perfectly until it comes to the one-base insertion or deletion. From that point on, the three-letter words in the message are one letter out of regis-



Sickle-cell phenotype Normal phenotype

72.7 7 Sick led and Normal Red Blood Cells

The misshapen red blood cell on the left is caused by a mutation that substitutes an incorrect amino acid in one of the two polypeptides of hemoglobin.

ter. In other words, such mutations shift the "reading frame" of the genetic message. Frame-shift mutations almost always lead to the production of nonfunctional proteins.

Chromosomal mutations are extensive changes in the genetic material

DNA molecules can break and rejoin, grossly disrupting the sequence of genetic information. There are four types of such chromosomal mutations: deletions, duplications, inversions, and translocations (Figure 12.18). These mutations can be caused by severe damage to chromosomes resulting from chemical or radiation exposure or by drastic errors in chromosome replication.

Deletions remove part of the genetic material (Figure 12.18a). Like frame-shift point mutations, their consequences can be severe unless they affect unnecessary genes or are masked by the presence, in the same cell, of normal alleles of the deleted genes. It is easy to imagine one mechanism that could produce deletions: A DNA molecule might break at two points, and the two end pieces might rejoin, leaving out the DNA between the breaks.

Another mechanism by which deletion mutations might arise would lead simultaneously to the production of a second kind of chromosomal mutation: a duplication (Figure 12.18b). Duplication would arise if homologous chromosomes broke at different positions and then reconnected to the wrong partners. One of the two molecules produced by this mechanism would lack a segment of DNA (it would have a deletion), and the other would have two copies (a duplication) of the segment that was deleted from the first.

Breaking and rejoining can also lead to inversion—the removal of a segment of DNA and its reinsertion into the same location, but "flipped" end over end so that it runs in the opposite direction (Figure 12.18c). If an inversion includes part of a segment of DNA that codes for a protein, the resulting protein will be drastically altered and almost certainly nonfunctional.

236 CHAPTER TWELVE

72.78 Chromosomal Mutations

Chromosomes may break during replication, and parts of chromosomes may then rejoin incorrectly. Letters on the colored chromosomes distinguish segments and identify consequences of duplications, deletions, inversions, and reciprocal translocations.

The fourth type of chromosomal mutation, called translocation, results when a segment of DNA breaks off, moves from a chromosome, and is inserted into a different chromosome. Translocations may be reciprocal, as in Figure 12.18d, or nonreciprocal, as the mutation involving duplication and deletion in Figure 12.18b illustrates. Translocations can make synapsis in meiosis difficult and thus sometimes lead to aneuploidy (a lack or excess of chromosomes; see Chapter 9).

Mutations can be spontaneous or induced

Spontaneous mutations are permanent changes in the genome that occur without any outside influence. In other words, they occur simply because the machinery of the cell is imperfect. They may occur by several mechanisms:

► The four nucleotide bases are somewhat unstable and can exist in two different forms (called tautomers), one of which is common and one rare. When a base temporarily forms its unusual tautomer, it can pair with a different base. For example, C normally pairs with G. But if C is in its rare form at the time of DNA replication, it pairs with (and DNA polymerase will

insert) A. So there is a mutation of G → A (Figure 2.9a, c).

► DNA polymerase makes errors in replication (see Chapter 11); for example, inserting a T opposite a G. Most of these errors are repaired by the proofreading function of the replication complex, but some errors escape and become permanent.

► Meiosis is not perfect. Nondisjunction can occur, leading to one too many or one too few chromosomes (aneuploidy; see Figure 9.19). Random chromosome breaks and rejoining among nonhomologous chromatids can occur, leading to translocations.

Induced mutations occur when some outside agent causes a permanent change in DNA:

► Some chemicals can covalently alter the nucleotide bases. For example, nitrous acid and its relatives can turn cytosine in DNA into uracil by deamination: It converts an amino group on cytosine (—NH₂) into a keto group

(—C=O). This changes the base pairing properties: C still pairs with G, but when U is present, DNA polymerase inserts an A. (Figure 12.19b, c).

► Other chemicals add groups to the bases. For instance, benzpyrene, a component of cigarette smoke (see page 199), adds a large chemical group to guanine, making it unavailable for base pairing. When DNA polymerase reaches such a modified guanine, it inserts any of the four bases; of course, three-fourths of the time the base will not be cytosine, and a mutation results.

Deletion is the loss of a chromosome segment

(b)

(c)

A B C D E F G

A B E F G

\

J (lost)

Duplication and deletion result when homologous chromosomes break at different points...

jv

A B C D E F G

..and swap segments. J

A B E F G

A B C D E F G

t



«>1 MM-)

Inversion results when a broken segment is inserted in reverse order.

(d)

A B C D E F G

A B E D C F G

Rediprocal translocation between nonhomologous chromosomes results when they exchange segments.



A B C D E F G

H I J K L M N O

H I J K C D E F G

► Radiation damages the genetic material in two ways. Ionizing radiation (X rays) produces highly reactive chemical species called free radicals, which can change bases in DNA to unrecognizable (by DNA polymerase) forms or break the sugar-

phosphate backbone, causing chromosomal abnormalities. Ultraviolet radiation from the sun (or a tanning lamp) is absorbed by thymine in DNA, causing it to form interbase covalent bonds with adjacent nucleotides. This too creates havoc with DNA replication.

Mutations have both benefits and costs. Mutations provide genetic diversity for evolution to work on, as we will see below. But they can also produce an organism that does poorly in its environment. An additional cost of mutations is that they can occur in non-gametes. Such somatic mutations can lead to cancer. We will return to the effects of germ-line and somatic mutations in humans in Chapter 18.

Mutations are the raw material of evolution

Without mutation, there would be no evolution. As we will see in Part Three of this book, mutation does not drive evolution, but it provides the genetic diversity on which natural selection and other agents of evolution act.

All mutations are rare events, but mutation frequencies vary from organism to organism and from gene to gene within a given organism. The frequency of mutation is usually much lower than one mutation per 10⁴ base pairs per DNA duplication, and sometimes as low as one mutation per 10⁹ base pairs per duplication. Most mutations are point mutations in which one nucleotide is substituted for another during the synthesis of a new DNA strand.

Mutations can harm the organism that carries them, or be neutral (have no effect on the organism's ability to sur-

(a) A spontaneous mutation

DNA

(b) An induced mutation

H

I N.

This C cannot hydrogen bond with G but instead pairs with A.

JN

DNA

N

H

Deamination by

O

HNCL

,N

u

DNA

N

O

O

Deaminated form of cytosine (= uracil)

This base cannot pair with G but instead pairs with A.

(c) The consequences of either mutation

§Q The mutated C usually reverts back to normal C, either spontaneously (if tautomeric) or by a repair system (if uracil)...

IA spontaneous or induced mutation of C occurs.



Original sequence

72.79 Spontaneous and Induced Mutations

(a) All four nitrogenous bases exist in both the prevalent common form and in a rare tautomeric form. When a base spontaneously forms its unusual tautomer, it can pair with a different base. (6) Mutagenic chemicals such as nitrous acid can induce changes in the bases, (c) In both spontaneous and induced mutations, the result is a permanent change in the DNA sequence.

vive or produce offspring). Once in a while, however, a mutation improves an organism's adaptation to its environment, or becomes favorable when environmental variables change.

Most of the complex creatures living on Earth have more DNA, and therefore more genes, than the simpler creatures do. Humans, for example, have 1,000 times more genetic material per cell than prokaryotes have. How did these new genes arise? If whole genes were sometimes duplicated by the mechanisms described in the previous section, the bearer of the duplication would have a surplus of genetic information that might be turned to good use. Subsequent mutations in one of the two copies of the gene might not have an adverse effect on survival, because the other copy of the gene would continue to produce functional protein. The extra gene might mutate over and over again without ill effect because its original function would be fulfilled by the original copy.

If the random accumulation of mutations in the extra gene led to the production of a useful protein (for example, an enzyme with an altered specificity for the substrates it binds, allowing it to catalyze different—but related—reactions), natural selection would tend to perpetuate the existence of this new gene. New copies of genes may also arise through the activity of transposable elements, which are discussed in Chapters 13 and 14.

AATGCTG TTACGAC

- ► Replication is normal

Template' strand

Chapter Summary

One Gene, One Polypeptide

- Genes are made up of DNA and are expressed in the phenotype as polypeptides (proteins).
- Beadle and Tatum's experiments with the bread mold *Neurospora* resulted in mutant strains lacking a specific enzyme in a biochemical pathway. These results led to the one-gene, one-polypeptide hypothesis. Review Figure 12.1
- Certain hereditary diseases in humans had been found to be caused by the absence of certain enzymes. These observations supported the one-gene, one-polypeptide hypothesis.

DNA, RNA, and the Flow of Information

- RNA differs from DNA in three ways: It is single-stranded, its sugar molecule is ribose rather than deoxyribose, and its fourth base is uracil rather than thymine.
- The central dogma of molecular biology is DNA → RNA → protein. Review Figure 12.2
- A gene is expressed in two steps: First, DNA is transcribed to RNA; then RNA is translated into protein. Review Figure 12.3
- In retroviruses, the rule for transcription is reversed: RNA → DNA. Other RNA viruses exclude DNA altogether, going directly from RNA to protein. Review Figure 12.2

Transcription: DNA-Directed RNA Synthesis

- RNA is transcribed from a DNA template after the bases of DNA are exposed by unwinding of the double helix.
- In a given region of DNA, only one of the two strands (the template strand) can act as a template for transcription.
- RNA polymerase catalyzes transcription from the template strand of DNA.

238 CHAPTER TWELVE

- The initiation of transcription requires that RNA polymerase recognize and bind tightly to a promoter sequence on DNA.
- RNA elongates in a 5'-to-3' direction, antiparallel to the template DNA. Special sequences and protein helpers terminate transcription. Review Figure 12.4

The Genetic Code

- The genetic code consists of triplets of nucleotides (codons). Since there are four bases, there are 64 possible codons.

► One mRNA codon indicates the starting point of translation and codes for methionine. Three stop codons indicate the end of translation. The other 60 codons code only for particular amino acids.

► Since there are only 20 different amino acids, the genetic code is redundant; that is, there is more than one codon for certain amino acids. But the code is not ambiguous: A single codon does not specify more than one amino acid. Review Figure 12.5

Preparation for Translation: Linking RNA's, Amino Acids, and Ribosomes

► In prokaryotes, translation begins before the mRNA is completed. In eukaryotes, transcription occurs in the nucleus and translation occurs in the cytoplasm.

► Translation requires four components: amino acids, tRNA's, activating enzymes, and ribosomes.

► In translation, amino acids are linked in an order specified by the codons in mRNA. This task is achieved by an adapter, transfer RNA (tRNA), which binds the correct amino acid and has an anticodon complementary to the mRNA codon. Review Figure 12.7

► The aminoacyl-tRNA synthetases, a family of activating enzymes, attach specific amino acids to their appropriate tRNA's, forming charged tRNA's. Review Figure 12.8

► The mRNA meets the charged tRNA's at a ribosome. Review Figure 12.9

Translation: RNA-Directed Polypeptide Synthesis

► An initiation complex consisting of an amino acid-charged tRNA and a small ribosomal subunit bound to mRNA triggers the beginning of translation. Review Figure 12.10

► Polypeptides grow from the N terminus toward the C terminus. The ribosome moves along the mRNA one codon at a time. Review Figure 12.11

► The presence of a stop codon in the A site of the ribosome causes translation to terminate. Review Figure 12.12

Regulation of Translation

► Some antibiotics work by blocking events in translation. Review Table 12.2

► In a polysome, more than one ribosome moves along the mRNA at one time. Review Figure 12.13

Posttranslational Events

► Signals contained in the amino acid sequences of proteins direct them to their cellular destinations. Review Figure 12.14

► Protein synthesis begins on free ribosomes in the cytoplasm. Those proteins destined for the nucleus, mitochondria, and plastids are completed there and have signals that allow them to bind to and enter their destined organelles.

► Proteins destined for the ER, Golgi apparatus, lysosomes, and outside the cell complete their synthesis on the surface

of the ER. They enter the ER by the interaction of a hydrophobic signal sequence with a channel in the membrane. Review Figure 12.15

► Covalent modifications of proteins after translation include proteolysis, glycosylation, and phosphorylation. Review Figure 12.16

Mutations: Heritable Changes in Genes

► Mutations in DNA are often expressed as abnormal proteins. However, the result may not be easily observable phenotypic changes. Some mutations appear only under certain conditions, such as exposure to a certain environmental agent (such as a drug) or condition (such as temperature).

► Point mutations (silent, missense, nonsense, or frame-shift) result from alterations in single base pairs of DNA. Review Pages 234-235

► Chromosomal mutations (deletions, duplications, inversions, or translocations) involve large regions of a chromosome. Review Figure 12.18

► Mutations can be spontaneous or induced. Spontaneous mutations occur because of instabilities in DNA or chromosomes. Induced mutations occur when an outside agent, such as a chemical or radiation, damages DNA. Review Figure 12.19

For Discussion

1. The genetic code is described as redundant. What does this mean? How is it possible that a point mutation, consisting of the replacement of a single nitrogenous base in DNA by a different base, might not result in an error in protein production?
2. Har Gobind Khorana, at the University of Wisconsin, synthesized artificial mRNA's such as poly CA (C AC AC A...) and poly

CAA (C A A C A A C A A...). He found that poly CA codes for a polypeptide consisting of threonine (Thr) and histidine (His) in alternation (His—Thr—His—Thr...). There are two possible codons in poly CA, CAC and ACA. One of these must code for histidine and the other for threonine—but which is which? The answer comes from results with poly CAA, which produces three different polypeptides: poly Thr, poly Gln (glutamine), and poly Asn (asparagine). (An artificial messenger can be read, inefficiently, beginning at any point in the chain; there is no specific start codon.) Thus poly CAA can be read as a polymer of CAA, of ACA, or of AAC. Compare the results of the poly CA and poly CAA experiments, and determine which codon codes for threonine and which for histidine.

3. Look back at Question 2. Using the genetic code (Figure 12.5) as a guide, deduce what results Khorana would have obtained had he used poly UG and poly UGG as artificial messengers. In fact, very few such artificial messengers would have given useful results. For an example of what could happen, consider poly CG and poly CGG. If poly C were the messenger, a mixed polypeptide of arginine and alanine (Arg—Ala—Ala—Arg...) would be obtained; poly CGG would give three polypeptides: poly Arg, poly Ala, and poly Gly (glycine). Can any codons be determined from only these data? Explain.

4. Errors in transcription occur about 100,000 times as often as do errors in DNA replication. Why can this high rate be tolerated in RNA synthesis but not in DNA synthesis?



The Genetics of Viruses and Prokaryotes



Janet, a member of her university's cross-country team, entered the hospital just after final exams for some long-delayed surgery on a tendon in her knee. The tendon repair went well, but she left the hospital with something new: bacteria called *Pseudomonas aeruginosa* had infected the surgical wounds. The antibiotics typically used to kill these bacteria did not work. She ended up back in the hospital two weeks later, where she received intensive antibiotic therapy and ultimately recovered.

Janet developed what is called a nosocomial infection—an infection acquired as a result of a hospital stay. Why would a hospital, which we think of as a place to get better, sometimes—in fact, for about 10 percent of all patients—be a place where we get sick? Of course, the stresses of Janet's surgery could have reduced her immunity to the bacteria that are common everywhere in our environment. Increasingly, however, the heavy use of antibiotics in hospitals makes them breeding grounds for bacteria that have genes for resistance to those antibiotics.

How have bacteria acquired antibiotic resistance so rapidly, and how do they pass that acquired resistance along to other bacteria? The answer involves some DNA sequences called R factors. But before we can discuss these remarkable pieces of DNA, we must introduce the genetics of prokaryotes. Prokaryotes usually reproduce asexually by cell division, but can acquire new genes in several ways. These range from simple recombination in a sexual process to using infective viruses as carriers for prokaryotic genes. We also describe how the expression of prokaryotic genes is regulated, and what DNA sequencing has revealed about the prokaryotic genome.

Viruses are not prokaryotes. In fact, they are not even cells, but intracellular parasites that can reproduce only within living cells. We begin this chapter by examining the structures, classification, reproduction, and genetics of viruses.

Using Prokaryotes and Viruses to Probe the Nature of Genes

Prokaryotes such as *Escherichia coli* and the viruses that infect them have been important tools in discovering

Are There Uninvited Guests Here?

A masked team performs surgery on a patient. But have harmful, drug-resistant bacteria invaded the surgical suite?

the structure, function, and transmission of genes, as we saw in Chapter 11. What are the advantages of working with prokaryotes and viruses?

First, it is easier to work with small amounts of DNA than with large amounts. A typical bacterium contains about a thousandth as much DNA as a single human cell, and a typical bacterial virus contains about a hundredth as much DNA as a bacterium. Second, data on large numbers of individuals can be obtained easily from prokaryotes. A single milliliter of medium can contain more than 10^9 *E. coli* cells or 10^{11} bacteriophages. In addition, most prokaryotes grow rapidly. A culture of *E. coli* can be grown under conditions that allow their numbers to double every 20 minutes. By contrast, 109 mice would cost more than 109 dollars, would require a cage that would cover about 3 square miles, and growing a generation of mice takes about 3 months, not 20 minutes. Third, prokaryotes and viruses are usually haploid, which makes genetic analyses easier.

The ease of growing and handling bacteria and their viruses permitted the explosion of genetics and molecular biology that began shortly after the mid-twentieth century. Their relative biological simplicity contributed immeasurably to discoveries about the genetic material, the replication of DNA, and the mechanisms of gene expression. Later, they were the first subjects of recombinant DNA technology (see Chapter 16).

Questions of interest to all biologists continue to be studied in prokaryotes, and prokaryotes continue to be important tools for biotechnology and for research on eukaryotes. Prokaryotes are important players in the environment, performing much of the cycling of elements in the atmosphere



240 CHAPTER THIRTEEN

and water. And, as we saw at the opening of this chapter, infectious diseases caused by prokaryotes and viruses continue to challenge humankind.

Viruses: Reproduction and Recombination

Although there are many kinds of viruses, most of them are composed of nothing but nucleic acid and a few proteins. Most viruses have relatively simple means of infecting their host cells. Some can infect a cell but postpone reproduction, lying low in the host chromosome until conditions are favorable. The simplest infective agents of all are viroids, which are made up only of genetic material.

Scientists studied viruses before they could see them

Most viruses are much smaller than most bacteria (Table 13.1). Viruses have become well understood only within the last half century, but the first step on this path of discovery was taken by the Russian botanist Dmitri Ivanovsky in 1892. He was trying to find the cause of tobacco mosaic disease, which results in the destruction of photosynthetic tissues and can devastate a tobacco crop. Ivanovsky passed an extract of diseased tobacco leaves through a fine porcelain filter, a technique that had been used previously by physicians and veterinarians to isolate disease-causing bacteria.

To Ivanovsky's surprise, the disease agent in this case did not stick to the filter: It passed through, and the liquid filtrate still caused tobacco mosaic disease. But instead of concluding that the agent was smaller than a bacterium, he assumed that his filter was faulty. Pasteur's recent demonstration that bacteria could cause disease was the dominant idea at the time, and Ivanovsky chose not to challenge it. But, as often happens in science, someone soon came along who did. In 1898, Martinus Beijerinck repeated Ivanovsky's experiment, and also showed that the tiny tobacco mosaic agent could diffuse through an agar gel. He called the agent *contagium vivum fluidum*, which later became shortened to virus.

Almost 40 years later, the infective agent was crystallized by Wendell Stanley (who won the Nobel Prize for his efforts). The crystalline viral preparation became infectious again when it was dissolved. It was soon shown that crys-

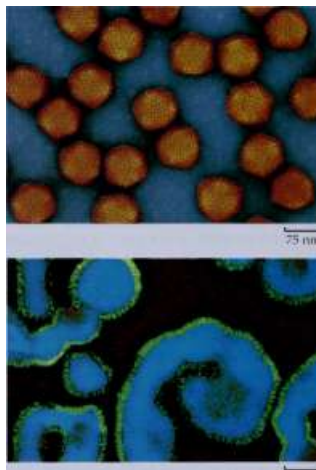


(a)

75 nm

(b)

(c)



20 nm

13.1 Virions Come in Various Shapes

(a) The tobacco mosaic virus (a plant virus) consists of an inner helix of RNA covered with a helical array of protein molecules. (b) Many animal viruses, such as this adenovirus, have an icosahedral (20-sided) capsid as an outer shell. Inside the shell is a spherical mass of proteins and DNA. (c) Not all virions are regularly shaped. Wormlike virions of the influenza A virus infect humans, causing chills, fever and sometimes, death.

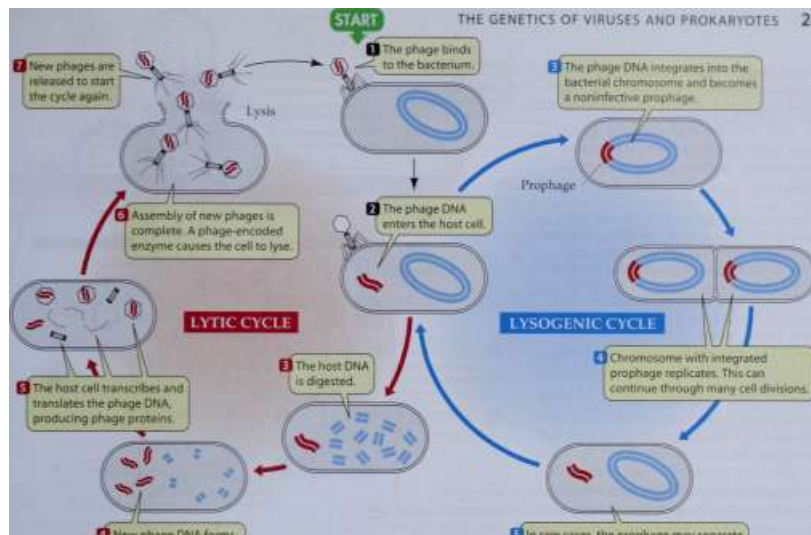
tallized viral preparations consist of protein and nucleic acid. Finally, direct observation of viruses with electron microscopes in the 1950s showed clearly how much they differ from bacteria and other organisms

Viruses reproduce only with the help of living cells

Unlike the organisms that make up the six taxonomic kingdoms of the living world, viruses are acellular; that is, they are not cells and do not consist of cells. Unlike cellular creatures, viruses do not metabolize energy—they neither produce ATP nor conduct fermentation, cellular respiration, or photosynthesis.

Whole viruses never arise directly from preexisting viruses. Viruses are obligate intracellular parasites; that is, they

Q The phage DNA integrates into the bacterial chromosome and becomes a noninfective prophage.



New phage DNA forms, using nucleotides from former host DNA.

develop and reproduce only within the cells of specific hosts. The cells of animals, plants, fungi, protists, and prokaryotes (both bacteria and archaea) serve as hosts to viruses. When they reproduce, viruses usually destroy the host cell, releasing progeny viruses that then seek new hosts.

Many diseases of humans, animals, and plants are caused by viruses. Because they lack the distinctive cell wall and ribosomal biochemistry of bacteria, viruses are not affected by antibiotics.

Viruses outside of host cells exist as individual particles called virions. The virion, the basic unit of a virus, consists of a central core of either DNA or RNA (but not both) surrounded by a capsid, or coat, composed of one or more proteins. The way in which these proteins assemble gives each type of virion a characteristic shape (Figure 13.1). In addition, many animal viruses have a lipid and protein membrane acquired from host cell plasma membranes.

There are many kinds of viruses

A common way to classify viruses separates them by whether their genetic material is DNA or RNA, and then by whether their nucleic acid is single-stranded or double-stranded. Some RNA viruses have more than one molecule of RNA, and the DNA of one virus family is circular. Further levels of classification depend on factors such as the overall shape of the virus and the symmetry of the capsid (see Figure 13.1). Another level of classification is based on the presence or absence of a membranous envelope around the virion; still further subdivision is based on capsid size.

Q In rare cases, the prophage may separate I and the cell will enter the lytic cycle.

73.2 The Lytic and Lysogenic Cycles of Bacteriophages

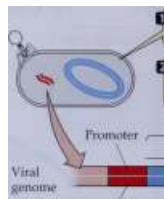
In the lytic cycle, infection by viral DNA leads directly to the multiplication of the virus and lysis of the host bacterial cell. In the lysogenic cycle, a prophage is replicated as part of the host's chromosome.

One way to classify viruses is based on the type of host. Let's see how reproductive cycles and other properties vary among the major groups of viruses: those that infect bacteria, animals, and plants.

Bacteriophages reproduce by a lytic cycle or a lysogenic cycle

Viruses that infect bacteria are known as bacteriophages. Bacteriophages recognize their hosts by means of specific binding between proteins in the capsid and receptor proteins in the host's cell wall. The virions, which must penetrate the cell wall, are often equipped with tail assemblies that insert their nucleic acid through the cell wall into the host bacterium. After the phage has injected its nucleic acid into the host, one of two things happens, depending on the kind of phage.

We saw one type of viral reproductive cycle when we studied the Hershey-Chase experiment (see Figure 11.3). That was the lytic cycle, so named because the infected bacterium lyses (bursts), releasing progeny phages. In the lysogenic cycle, the infected bacterium does not lyse, but instead harbors the viral nucleic acid for many generations. Some viruses reproduce only by the lytic cycle; others undergo both types of reproductive cycles (Figure 13.2).



Viral genome

?

242 CHAPTER THIRTEEN

the lytic cycle. A phage that reproduces only by the lytic cycle is called a virulent virus. Once the phage has injected its nucleic acid into the host cell, the phage nucleic acid takes over the host's synthetic machinery. It does so in two stages (Figure 13.3):

- The early stage transcribes the virus's early genes. This part of the viral genome contains the promoter sequence that attracts host RNA polymerase. The early genes often include proteins that shut down host transcription, stimulate viral genome replication, and stimulate late gene transcription. Nuclease enzymes digest the host chromosome, providing nucleotides for the synthesis of viral genomes.
- The late stage transcribes the virus's late genes. These genes code for the proteins that package virions and lyse the host cell to release the new virions.

This sequence of transcriptional events is carefully controlled: Premature lysis of the host cell before virus particles are ready for release would stop the infection. The whole process—from binding and infection to lysis of the host—takes about half an hour.

Rarely, two viruses infect a cell at the same time. This is an unusual event, as once an infection cycle is under way, there is usually not enough time for an additional infection. In addition, an early gene product prevents further infections in some cases. The presence of two viral genomes in the same host cell affords the opportunity for genetic recombination by crossing over, as in prophase of meiosis. This enables genetically different viruses of the same kind to swap genes and create new strains.

the lysogenic cycle. Phage infection does not always result in lysis of the host cell. Some phages seem to disappear from a bacterial culture, leaving the bacteria immune to further attack by the same strain of phage. In such cultures, however, a few free phages are always present. Bacteria harboring phages that are not lytic are called lysogenic, and the phages are called temperate viruses.

Lysogenic bacteria contain a noninfective entity called a prophage: a molecule of phage DNA that has been integrated into the bacterial chromosome (see Figure 13.2). As part of the bacterial genome, the prophage can remain quiet within the bacteria through many cell divisions. However, an occasional lysogenic bacterium can be induced to activate its prophage. This results in a lytic cycle, releasing a large number of free phages, which can then infect other uninfected bacteria and renew the reproductive cycle.

This capacity to switch between the lysogenic and the lytic cycle is very useful to the phage, whose purpose is to reproduce as many offspring as possible. When its host cell is growing slowly and is low on energy, the phage becomes

A virus infects a host cell.

fj| It uses the host cell's RNA polymerase to transcribe early genes.

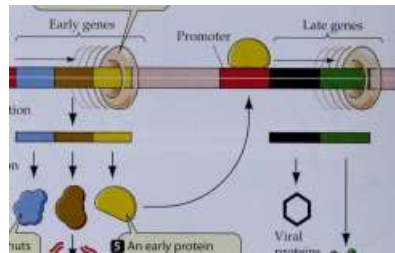
Early genes

Late genes

Transcription mRNA

Translation X

Q An early protein shuts down host gene transcription...



'J*

I An early protein stimulates late gene transcription...

Viral proteins

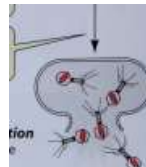
...and another stimulates viral genome reproduction.

t

leading to production new viral structures...

*

Lysis proteins



Q ...and a protein that lyses the host cell, releasing virus particles

13.3 A Strategy for DNA Virus Reproduction

In a host cell infected with a virulent virus, the

viral genome shuts down host transcription

while it replicates itself. Once the viral genome

is replicated, its "late" genes produce proteins that "package" the

genome and then lyse the host cell.

lysogenic. Then, when the host's health is restored to a level that provides maximal resources for phage reproduction, the prophage is released from its dormant state, and the lytic cycle proceeds. We will see how this switch works later in the chapter when we discuss control of gene expression.

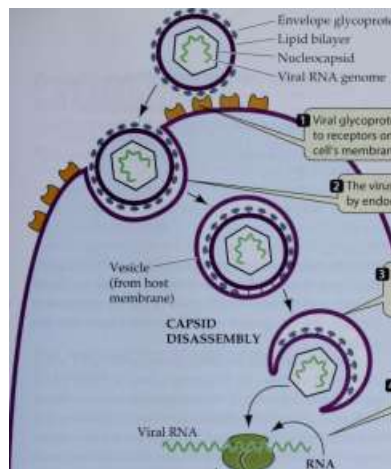
Animal viruses have diverse reproductive cycles

Almost all vertebrates are susceptible to viral infections, but among invertebrates, such infections are common only in arthropods (the group that includes insects and crustaceans). One group of viruses, called arboviruses (short for "arthropod-borne viruses"), is transmitted to a mammalian host through an insect bite. Although carried within the arthropod host's cells, arboviruses apparently do not affect that host severely; they affect only the bitten and infected mammal. The arthropod acts as a vector—an intermediate carrier—transmitting the disease organism from one host to another.

Animal viruses are very diverse. Some are just particles of proteins surrounding a nucleic acid core. Others have a membrane derived from the host cell's plasma membrane. Some animal viruses have DNA as their genetic material; others have RNA. In all cases, the small viral genome has limited coding capacity, making only a few proteins.

Envelope glycoprotein Lipid bilayer Nucleocapsid Viral RNA genome

Viral glycoproteins bind to receptors on the host cell's membrane.



* Virus

THE GENETICS OF VIRUSES AND PROKARYOTES 243

73.4 The Reproductive Cycle of the Influenza Virus

The membrane-enclosed, or enveloped, influenza virus is taken into the host cell by endocytosis. Once inside, fusion of the vesicle and viral membranes releases the RNA genome, which replicates and assembles new virions.

The virus enters the cell by endocytosis.

Viral and vesicle membranes fuse, releasing the virion.

I

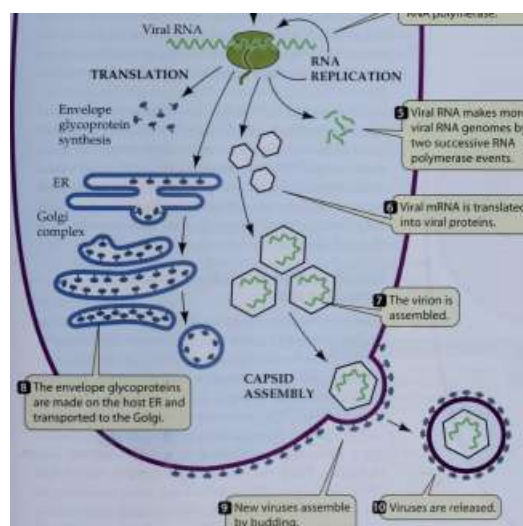
Viruses with membranes (called enveloped viruses) may also be taken up by endocytosis (see Figure 13.4), and released from a vesicle. In these viruses, the viral membrane is studded with glycoproteins that bind to receptors on the host cell plasma membrane. More commonly, the host and viral membranes fuse, releasing the rest of the virion into the cell (see Figure 13.5).

Viral RNA makes mRNA via viral RNA-dependent RNA polymerase.

k_J RNA

f I i^— REPLICATION

Viral RNA makes more viral RNA genomes by two successive RNA polymerase events.



Like that of bacteriophages, the life cycle of animal viruses can be divided into early and late stages (see Figure 13.3). Animal viruses enter cells in one of three ways:

► A naked virion (without a membrane) is taken up by endocytosis, which traps it within a membranous vesicle inside the host cell. The membrane of the vesicle breaks down, releasing the virion into the cytoplasm, and the host cell digests the protein capsid, liberating the viral nucleic acid, which takes charge of the host cell.

Enveloped viruses usually escape from the host cell by budding through virus-modified areas of the host's plasma membrane. During this process, the completed virions acquire a membrane similar to that of the host cell.

The life cycles of influenza virus and HIV, two important RNA viruses, illustrate the two different styles of infection by enveloped viruses. Influenza virus is endocytosed into a membrane vesicle (Figure 13.4). Fusion of the viral and vesicle membranes releases the virion into the cell. The virus carries its own enzyme to replicate its RNA genome into a complementary strand. The latter is then used as mRNA to make, by complementary base pairing, more copies of the viral genome.

Retroviruses such as HIV have a more complex reproductive cycle (Figure 13.5). The virus enters a host cell by direct fusion of viral and cellular membranes. A major feature of the retroviral life cycle is the reverse transcription of retroviral RNA to produce a DNA provirus (cDNA), which is the form of the viral genome that gets integrated into the host DNA. The provirus may reside in the host chromosome permanently, occasionally being expressed to produce new virions. Almost every step in this complex cycle can, in principle, be attacked by therapeutic drugs; this fact is used by researchers in their quest to conquer AIDS, the deadly condition caused by HIV infection in humans. This medical battle will be discussed further in Chapter 19.

Many plant viruses spread with the help of vectors

Viral diseases of flowering plants are very common. Plant viruses can be transmitted horizontally, from one plant to another, or vertically, from parent to offspring. To infect a plant cell, viruses must pass through a cell wall and through the host plasma membrane. Most plant viruses accomplish this penetration through their association with

244 CHAPTER THIRTEEN

Q HIV attaches to host cell at membrane protein CD4.

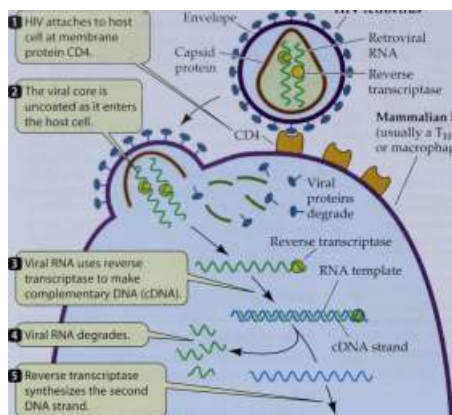
HIV retrovirus

Retroviral RNA

Reverse transcriptase

Mammalian host

(usually a T_H cell or macrophage)



cell

73.5 The Reproductive Cycle of HIV

The retrovirus HIV enters a host cell via fusion of its membranes with the host's plasma membrane. Reverse transcription of retroviral RNA then produces a DNA provirus—a strand of complementary DNA that enters the host nucleus, where it transcribes viral RNA.

Q Viral RNA degrades.

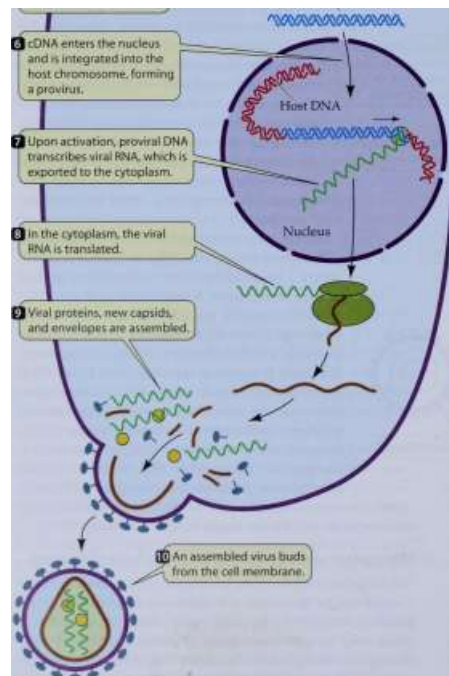
" I

o Reverse transcriptase synthesizes the second DNA strand.

r ^omomom(K

Q cDNA enters the nucleus and is integrated into the host chromosome, forming a provirus.

Q Upon activation, proviral DNA transcribes viral RNA, which is exported to the cytoplasm.



vectors. Infection of a plant usually results from attack by a virion-laden insect vector. When an insect vector penetrates a cell wall with its proboscis (snout), the virions can move from the insect into the plant.

Plant viruses can be introduced artificially, without insect vectors, by bruising a leaf or other plant part, then exposing it to a suspension of virions. Horizontal viral infections may also occur in nature if a bruised infected plant contacts an injured uninfected one. Vertical transmission of viral infections may occur through vegetative or sexual reproduction.

Once inside a plant cell, the virus reproduces and spreads to other cells in the plant. Within an organ such as a leaf, the virus spreads through the plasmodesmata, the cytoplasmic connections between cells. Because the viruses are too large to go through these channels, special proteins bind to them and help change their shape so that they can squeeze through the pores.

Viroids are infectious agents consisting entirely of RNA

Pure viral nucleic acids can produce viral infections under laboratory conditions, although inefficiently. Might there be infectious agents in nature that consist of nucleic acid without a protein capsid? In 1971, Theodore Diener of the U.S. Department of Agriculture reported the isolation of agents of this type, called viroids. Viroids are circular, single-stranded RNA molecules consisting of a few hundred nucleotides. They are one-thousandth the size of the smallest viruses. These RNA's are most abundant in the nuclei of infected cells. Viroids have been found only in plants, in which they produce a variety of diseases. Like plant viruses, viroids can be transmitted horizontally or vertically.

There is no evidence that viroids are translated to synthesize proteins, and it is not known how they cause disease. Viroids are replicated by the enzymes of their plant hosts. Similarities in base sequences between viroids and certain nontranslated sequences (introns) of plant genes suggest that viroids evolved from introns. This conclusion is supported by the fact that viroids, although made of RNA, are catalytically active in the way that some introns are.

Prokaryotes: Reproduction, Mutation, and Recombination

In contrast to viruses, bacteria and archaea are living cells. Prokaryotes carry out all the functions required for their own reproduction. They harvest and use energy, and they produce and use the molecular equipment that synthesizes their components and replicates their genes.

Prokaryotes usually reproduce asexually, but nonetheless have several ways of recombining their genes. Whereas in eukaryotes, genetic recombination occurs between the genomes of two parents, in prokaryotes it results from the interaction of the genome of one cell with a much smaller sample of genes from another cell.

The reproduction of prokaryotes gives rise to clones

Most prokaryotes reproduce by the division of single cells into two identical offspring (see Figure 9.3). In this way, a single cell gives rise to a clone—a population of genetically identical individuals. Prokaryotes reproduce very rapidly. A population of *E. coli*, for example, can double every 20 minutes, as long as conditions remain favorable.

Pure cultures of *E. coli* or other bacteria can be grown on the surface of a solid medium that contain a sugar, minerals, a nitrogen source such as ammonium chloride (NH_4Cl), and a solidifying agent such as agar (Figure 13.6). If the number of cells spread on the medium is small, each cell will give rise to a small, rapidly growing bacterial colony. If a large number of cells is poured onto the solid medium, their growth will produce one continuous colony—a bacterial "lawn." Bacteria can also be grown in a liquid nutrient medium. We'll see examples of all these techniques in this chapter.

Some bacteria conjugate, recombining their genes

The existence and heritability of mutations in bacteria attracted the attention of geneticists to these microbes. But if there were no form of exchange of genetic information between individuals, bacteria would not be useful for genetic analysis. Luckily, in 1946, Joshua Lederberg and Edward Tatum demonstrated that such exchanges do occur, although they are rare events.

Lederberg and Tatum grew two nutrient-requiring, or auxotrophic, strains of *E. coli*. Like the *Neurospora* in Figure 12.1, these strains will not grow on a minimal medium, but require supplementation with a nutrient that they cannot synthesize for themselves because of an enzyme defect. *E. coli* strain 1 requires the amino acid methionine and the vitamin biotin for growth, and its genotype is symbolized as *met⁻bio⁻*. Strain 2 requires neither of these substances, but cannot grow without the amino acids threonine and leucine. Considering all four factors, we say that strain 1 is *met⁻bio⁻thr⁺leu⁺* and strain 2 is *met⁺bio⁺thr⁻leu⁻*.

Lederberg and Tatum mixed these two mutant strains and cultured them together for several hours on a medium supplemented with methionine, biotin, threonine, and leucine, so that both strains could grow. The bacteria were then removed from the medium by centrifugation, washed, and transferred to minimal medium, which lacked all four supplements. Neither strain could grow on this medium because of their nutritional requirements. However, a few colonies did appear on the plates (Figure 13.7). Because they grew in the minimal medium, these colonies must have consisted of bacteria that were *met⁺bio⁺thr⁺leu⁺*; that is, they must have been prototrophic. These colonies appeared at a rate of approximately 1 for every 10 million cells originally put on the plates ($1/10^7$).

t

RESEARCH METHO

A solid nutrient medium is inoculated with a small number of bacteria.



f

A solid nutrient medium is inoculated with 10^8 - 10^9 bacteria.

f

A liquid nutrient medium is inoculated with bacteria.

One „/\".

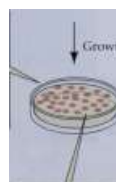
hour's < J\ „/\". growth I AililA

In a few hours of doubling populations, there will be millions of cells.

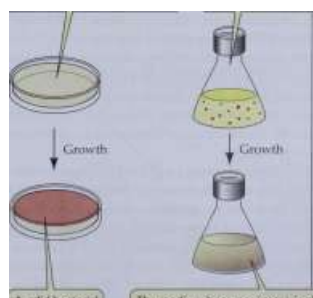
Growth

13.6 Growing Bacteria in the Laboratory

A population of *E. coli* doubles every 20 minutes in laboratory culture. The different techniques of culture shown are used for different applications.



A colony grows where each bacterium lands.



A solid bacteria "lawn" forms.

The medium becomes increasingly cloudy as the bacteria multiply.

EXPERIMENT

Question: Can bacteria exchange genetic material? When different auxotrophic (mutant) strains of bacteria are grown together, do new prototrophic (wild-type) bacteria appear?

Strain 1 of *E. coli* (met⁻ bio⁻ thr⁻ leu⁺) requires methionine and biotin for growth

Growth occurs on minimal medium methionine and biotin.



Strain 2 of *E. coli* (met⁺ bio⁺ thr⁻ leu⁻) requires threonine and leucine for growth

Growth occurs on minimal medium + threonine and leucine.



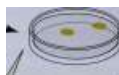
There is no growth on minimal medium.

Samples of strains 1 and 2 are combined and incubated together in liquid medium with methionine, biotin, threonine, and leucine.

RESULTS

Complete medium

(many colonies grow).



On minimal medium with no supplements, a few colonies of prototrophic bacteria (met⁺ bio⁺ thr⁺ leu⁺) grow.

Conclusion: The prototrophic colonies growing on minimal medium could have arisen only by genetic recombination.

73.7 Lederberg and Tatum's Experiment

After growing together, a mixture of complementary auxotrophic strains of *E. coli* contained a few cells that gave rise to new prototrophic colonies. This experiment proved that genetic recombination takes place in prokaryotes.

Where did these prototrophic colonies come from? Lederberg and Tatum were able to rule out mutation, and other investigators ruled out transformation. A third possibility is that the two strains of bacteria had exchanged genetic material, allowing it to mix and recombine to produce cells containing met⁺ and bio⁺ alleles from strain 2 and thr⁺ and leu⁺ alleles from strain 1 (see Figure 13.7). Later experiments showed that such an exchange, called conjugation, had indeed occurred.

One bacterial cell—the recipient —had received DNA from the other cell—the donor —that included the two wild-type alleles that were missing in the recipient. Recombination then created a genotype with four wild-type alleles.

The physical contact required for conjugation can be observed under the electron microscope (Figure 13.8). It is initiated by a thin projection called a pilus. Then the actual transfer of DNA from one cell to another occurs by a thin conjugation tube. Since bacterial DNA is circular, it must be made linear (broken) so that it can pass through the tube. Contact between the cells is brief—certainly not long enough for all of the donor genome to enter the recipient cell. Therefore, the recipient cell usually receives only a portion of the donor DNA.

Once the donor fragment is inside the recipient cell, recombination can occur. In much the same way that chromosomes pair up, gene for gene, in prophase of meiosis, the donor DNA can line up beside its homologous gene in the recipient. Enzymes that can cut and rejoin DNA molecules are active in bacteria, and so gene(s) of the donor can end up integrated into the genome of the recipient, thus changing its genetic constitution (Figure 13.9).



The two cells are connected by thin tubes called pili.



The tiny "beads" on the pili are bacteriophages that attach specifically to pili, making them more visible. They were added after conjugation.

13.8 Bacterial Conjugation

Pili draw two bacteria into close contact, and DNA is transferred from one cell to the other via a conjugation tube.

DNA

(from donor chromosome)

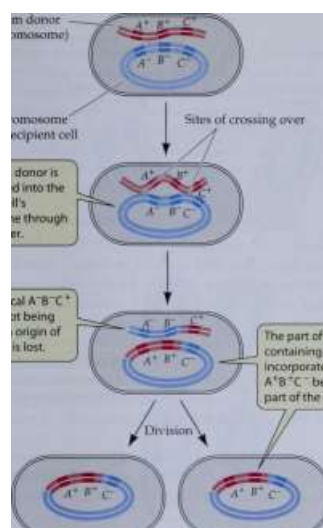
Chromosome of recipient cell

of crossing over

r

DNA from a donor is incorporated into the recipient cell's chromosome through crossing over.

The reciprocal A~B~C + segment, not being linked to an origin of replication, is lost.



The part of the donor chromosome containing the A + and B + genes is incorporated. The sequence A + B + C " becomes a permanent part of the recipient genotype.

inside a host cell, an event very similar to recombination occurs, and new genes can be incorporated into the host chromosome.

In transduction, viruses carry genes from one cell to another

When bacteriophages undergo a lytic cycle, they package their genomic DNA in capsids. These capsids generally form before the DNA is inserted into them. Sometimes, bacterial DNA fragments get inserted into the empty phage capsids instead of the phage DNA. (Figure 13.10fr).

Recall that the binding of a phage to its host cell and the insertion of phage DNA are carried out by the capsid. So, when a phage capsid carries a piece of bacterial DNA, the latter is injected into the "infected" bacterium. This mechanism of DNA transfer is called transduction. Needless to say, it does not result in a productive viral infection. Instead, the incoming DNA fragment can recombine with the host chromosome.

73.9 Recombination Following Conjugation

DNA from a donor cell may become incorporated into the recipient cell's chromosome through crossing over. Only about half the transferred genes become integrated in this way.

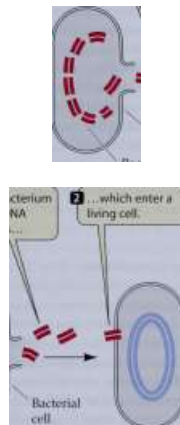
In transformation, cells pick up genes from their environment

Frederick Griffith obtained the first evidence for the transfer of prokaryotic genes more than 75 years ago when he discovered the transforming principle (see Figure 11.1). We now know the reason for his results: DNA had leaked from dead cells of virulent pneumococci and was taken up as free DNA by living nonvirulent pneumococci, which became virulent as a result. This phenomenon, called transformation, occurs in nature in some species of bacteria when cells die and their DNA leaks out (Figure 13.10fl). Once transforming DNA is

(a) Transformation

Q A lysed bacterium releases DNA fragments...

.which enter a living cell.



Q Recombination occurs between the DNA fragment and host chromosome.



Bacterial cell

Bacterial chromosome

II

(b) Transduction

t

Phage DNA is incorporated into host bacterial DNA.

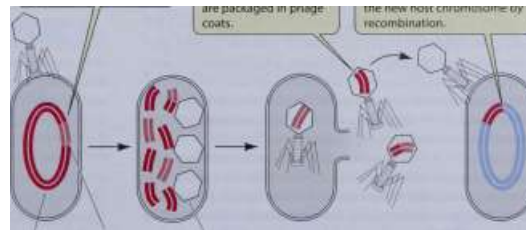
Ej During the lytic cycle, bacterial DNA fragments are packaged in phage coats.

o In a subsequent "infection," the bacterial DNA is inserted into the new host chromosome by recombination.

13.10 Transformation and Transduction

After a new DNA fragment enters the host cell, recombination can occur, (a) Transforming DNA can leak from dead bacterial

cells and be taken up by a living host bacterium, which may incorporate new genes into its chromosome, (b) In transduction, viruses carry DNA fragments from one cell to another.



Bacterial DNA

Phage DNA (prophage)

Phage coats

248 CHAPTER THIRTEEN

Plasmids are extra chromosomes in bacteria

In addition to their main chromosome, many bacteria harbor additional smaller, circular chromosomes. These chromosomes, called plasmids, contain at most a few dozen genes, and, importantly, an origin sequence where DNA replication starts, which defines them as chromosomes. Usually, plasmids replicate at the same time as the host chromosome during the bacterial cell cycle, but this is not necessarily the case.

Plasmids are not viruses. They do not take over the cell's molecular machinery or make a protein coat to help them move from cell to cell. Instead, they can move between cells during conjugation, thereby adding some new genes to the recipient bacterium (Figure 13.11). Since plasmids exist independently of the main chromosome (the term episomes is sometimes used), they do not need to recombine with the main chromosome to add their genes to the cell's genome.

There are several types of plasmids, classified according to the kinds of genes they carry.

METABOLIC FACTORS CARRY GENES FOR UNUSUAL METABOLIC FUNCTIONS. Some plasmids, called metabolic factors, have genes that allow their recipients to carry out unusual metabolic functions. For example, there are many unusual hydrocarbons in oil spills. Some bacteria can actually thrive on these molecules, using them as a carbon source. The genes for the enzymes involved in these degradative pathways are carried on plasmids.

F FACTORS CARRY GENES FOR CONJUGATION. The "F" in F factors

stands for fertility. Their approximately 25 genes include the ones that make both the pilus for attachment and the conjugation tube for DNA transfer to a recipient bacterium. A cell harboring the F factor is called F⁺. It can transfer a copy of the F factor to an F⁻ cell, making the recipient F⁺. Sometimes the factor integrates into the main chromosome (at which point it is no longer a plasmid), and when it does, it can bring along some bacterial genes when it moves through the conjugation tube from one cell to another.

r factors are resistance factors. R factors may carry genes coding for proteins that destroy or modify antibiotics. Other R factors provide resistance to heavy metals that bacteria encounter in their environment.

R factors first came to the attention of biologists in 1957 during an epidemic in Japan, when it was discovered that some strains of the *Shigella* bacterium, which causes dysentery, were resistant to several antibiotics. Researchers found that resistance to the entire spectrum of antibiotics could be transferred by conjugation even when no genes on the main chromosome were transferred.

Eventually it was shown that the genes for antibiotic resistance are carried on plasmids. Each R factor carries one or more genes conferring resistance to particular antibiotics, as well as genes that code for proteins involved in the transfer of DNA to a recipient bacterium. As far as biologists can determine, R factors appeared long before antibi-

Ori r

A plasmid has an origin of replication and genes for other functions

Bacterium with plasmids

Bacterium without plasmids

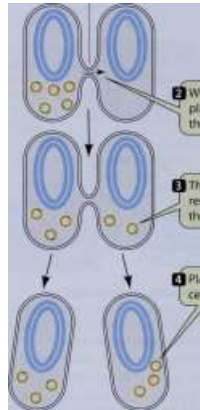


Bacterial chromosome



Plasmid

\ Conjugation / \ tube f



When bacteria conjugate, plasmids can pass through the conjugation tube.

| The recipient bacterium receives the plasmids and their genes.

Plasmids replicate as the host cell grows and divides.

73.7 7 Gene Transfer by Plasmids

When plasmids enter a cell via conjugation, their genes can be expressed in the new cell.

otics were discovered, but they seem to have become more abundant in modern times, possibly because the heavy use of antibiotics in the hospital environment selects for bacterial strains bearing them.

R factors also pose a threat to people in the general clinical setting if antibiotics are used inappropriately. You probably have gone to see a physician because of a sore throat, which can have either a viral or a bacterial cause. The best way to know is for the doctor to take a small sample from your inflamed throat, culture it, and identify any bacteria that are present. But perhaps you cannot wait another day for the results. Impatient, you ask the doctor to give you something to make you feel better. She prescribes an antibiotic, which you take. The sore throat gradually gets better, and you think that the antibiotic did the job.

But suppose the infection was viral? In that case, the antibiotic did nothing to combat the disease, which just ran its

normal course. However, it may have done something harmful: By killing many normal bacteria in your body, it may have exerted selection for bacteria harboring R factors. These bacteria may reproduce in the presence of the antibiotic, and may soon become quite numerous. The next time you got a bacterial infection, there would be a ready supply of R factors to be transferred to the invading bacteria, and antibiotics might be ineffective.

Transposable elements move genes among plasmids and chromosomes

As we have seen, plasmids, viruses, and even phage cap-sids (in the case of transduction) can transport genes from one bacterial cell to another. There is another type of "gene transport" that occurs within the individual cell. It relies on segments of chromosomal or plasmid DNA that can be inserted either at new locations on the same chromosome, or into another chromosome. These DNA sequences are called transposable elements. Their insertion often produces phe-notypic effects by disrupting the genes into which they are inserted (Figure 13.12fl).

The first transposable elements to be discovered in prokaryotes were large pieces of DNA, typically 1,000 to 2,000 base pairs long, found in many places in the E. coli

(a) j Transposable element

ABC

Jr

D

T

1

1

Copying and insertion



DNA

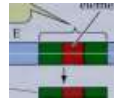
mRNA

If a transposable element is copied and inserted into the middle of another gene, the original gene can no longer be transcribed to yield an appropriate mRNA.

C

D

T



Copy of

transposable

element

DNA

Inappropriate mRNA

(b) Transposable Other genes Transposable

element element



DNA

Transposon ___J\

A transposon consists of two transposable elements flanking another gene or genes. The entire transposon is copied and inserted as a unit. It may disrupt a gene, or it may direct the actions of other genetic elements.

13.12 Transposable Elements and Transposons

(a) Transposable elements are segments of DNA that can be inserted at new locations, either on the same chromosome or on a different chromosome, (b) Transposons consist of transposable elements combined with other genes.

main chromosome. In one mechanism of transposition, the sequence of a transposable element replicates independently of the rest of the chromosome. The copy then inserts itself at other, seemingly random places in the chromosome. The genes encoding the enzymes necessary for this insertion are found within the transposable element itself. Some other transposable elements are cut from their original sites and inserted elsewhere without replication. Many transposable elements discovered later were longer (about 5,000 base pairs) and carried one or more additional genes with them. These longer elements with additional genes are called transposons (Figure 13.12b).

What do transposons and other transposable elements have to do with the genetics of prokaryotes—or with hospitals? Transposable elements have contributed to the evolution of plasmids. R plasmids probably originally gained their genes for antibiotic resistance through the activity of transposable elements. One piece of evidence for this conclusion is that each resistance gene in an R plasmid is part of a transposon.

Regulation of Gene Expression in Prokaryotes

Except for mutations, all cells of a bacterial species have the same DNA, and thus the capacity to make the same proteins. Yet the protein content of a bacterium can change rapidly when conditions warrant. For example, there are two ways for a bacterium to get the amino groups that it needs to make amino acids and proteins. One way involves taking N_2 from the air and "fixing" it into ammonia (NH_3), then using the ammonia as a source of amino groups. This reaction requires several enzymes and a lot of energy.

The other way to obtain amino groups is to take them from glutamine (see Table 3.2) and use them directly. This reaction requires only one enzyme and is not as endergonic. If there is a lot of glutamine around, the cell takes the easy way out, using the glutamine rather than the N_2 pathway. In fact, the enzymes that are involved in the N_2 pathway are not even made when glutamine is present.

There are several ways in which a prokaryotic cell could shut off the synthesis or activity of an unneeded protein:

- ▶ The cell could block the transcription of mRNA for that protein.
- ▶ The cell could hydrolyze the mRNA after it was made.
- ▶ The cell could prevent translation of the mRNA at the ribosome.
- ▶ The cell could hydrolyze the protein after it was made.

These methods would all have to be selective, responding to some biochemical signal. In the case of our two pathways for obtaining amino groups, the signal might be an increased concentration of glutamine.

Clearly, the earlier the cell intervenes in the process, the less energy it has to expend. Selective inhibition of transcription is far more efficient than transcribing the gene, translating the message, and then degrading the protein. While there are examples of all four methods of control of

250 CHAPTER THIRTEEN

0 10

Time after addition of inducer (minutes)

73.73 An Inducer Stimulates the Synthesis of an Enzyme

It is most efficient for a cell to produce an enzyme only when it is needed. Some enzymes are induced by the presence of the substance they act upon (for example, (3-galactosidase is induced by the presence of lactose).

protein levels in nature, prokaryotes generally use the most efficient one, transcriptional control.

Regulation of transcription conserves energy

As a normal inhabitant of the human intestine, *E. coli* has to adjust to sudden changes in its chemical environment. Its host may present it with one foodstuff one hour and another the next. This variability presents the bacterium with a metabolic challenge. Glucose is its preferred energy source, and is the easiest sugar to metabolize, but not all of its host's foods contain an abundant supply of glucose. For example, the bacteria may suddenly be deluged with milk, the main carbohydrate of which is the sugar lactose. Lactose is a (3-galactoside—a disaccharide containing galactose fi linked to glucose (see Chapter 3). Before lactose can be of any use to the bacteria, it must first be taken into the cells by a membrane transport carrier called (3-galactoside permease. Then it must be hydrolyzed to glucose and galactose by the enzyme P-galactosidase. A third protein, the enzyme thiogalactoside transacetylase, is also required for lactose metabolism.

When *E. coli* is grown on a medium that does not contain lactose or other P-galactosides, the levels of all three of these enzymes within the bacterial cell are extremely low—the cell does not waste energy and materials making the un-needed proteins. If, however, the environment changes such that lactose is the predominant sugar and very little glucose is

Regulation of enzyme activity

present, the synthesis of all three of these enzymes begins promptly, and they increase rapidly in abundance. For example, there are only two molecules of ^-galactosidase present in an *E. coli* cell when glucose is present in the medium. But when it is absent, lactose can induce the synthesis of 3,000 molecules of (3-galactosidase per cell!

If lactose is removed from *E. coli*'s environment, synthesis of the three enzymes that process it stops almost immediately. The enzyme molecules that have already formed do not disappear; they are merely diluted during subsequent growth and reproduction until their concentration falls to the original low level within each bacterium.

Compounds that stimulate the synthesis of an enzyme (such as lactose in our example) are called inducers (Figure 13.13). The enzymes that are produced are called inducible enzymes, whereas enzymes that are made all the time at a constant rate are called constitutive enzymes.

We have now seen two basic ways to regulate the rates of metabolic pathways. Chapter 6 described allosteric regulation of enzyme activity (the rate of enzyme-catalyzed reactions); this mechanism allows rapid fine-tuning of metabolism. Regulation of protein synthesis—that is, regulation of the concentration of enzymes—is slower, but produces a greater savings of energy. Figure 13.14 compares these two modes of regulation.

A single promoter controls the transcription of adjacent genes

The genes that serve as blueprints for the synthesis of the three proteins that process lactose are called structural genes, indicating that they specify the primary structure (the amino acid sequence) of a protein molecule. In other words, structural genes are those that can be transcribed into mRNA. Three such genes are involved in the metabolism of lactose, and they lie adjacent to each other on the *E. coli* chromosome. This is no coincidence. Their DNA is transcribed into a single, continuous molecule of mRNA. Because this particular messenger governs the synthesis of all three lactose-metabolizing enzymes, either all or none of

The end product feeds back, inhibiting the activity of enzyme 1 and quickly stopping the pathway.

Precursor

®

A

4

Gene 1

I Gene 2

◆

<S»

I

Gene 3

C

1

<S>

Gene 4

D

f

O

I Gene 5

End product

73. 14 Two Ways to Regulate a Metabolic Pathway

Feedback from the end product can block enzyme activity, or it can stop the transcription of genes that code for the enzyme.

Regulation of enzyme concentration

V

The end product blocks the transcription of all five genes. No enzymes are produced, the enzyme concentration falls, and the pathway stops.

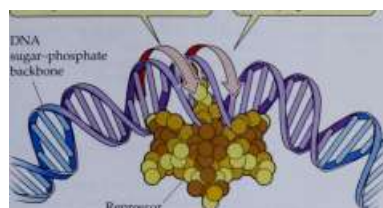
A portion of the repressor binds the minor groove of the DNA and...

...another portion binds the major groove.

DNA

sugar-phosphate

backbone



Repressor molecule

73.75 Repressor Bound to Operator Blocks Transcription

Portions of the repressor bind to the major and minor grooves in the DNA helix, preventing transcription.

the enzymes are made, depending on whether their common message—their mRNA—is present in the cell.

The three genes share a single promoter. Recall from Chapter 12 that a promoter is a site on DNA where RNA polymerase

binds to initiate transcription. The promoter for these three structural genes is very efficient, so that the maximum rate of mRNA synthesis can be high, but there must also be a way to shut down mRNA synthesis when the enzymes are not needed.

Operons are units of transcription in prokaryotes

Prokaryotes shut down transcription by placing an obstacle between the promoter and its structural genes. A short stretch of DNA called the operator lies in this position. It can bind very tightly to a special type of protein molecule, called a repressor, to create such an obstacle. When the repressor protein is bound to the operator region of DNA, it blocks the transcription of mRNA (Figure 13.15). When the repressor is not attached to the operator, mRNA synthesis proceeds rapidly.

The whole unit, consisting of the closely linked structural genes and the stretches of DNA that control their transcription, is called an operon (Figure 13.16). An operon al-

t

Operons have regulatory sequences that control the transcription of...

1 v

Regulatory sequences

ways consists of a promoter, an operator, and two or more structural genes. The promoter and operator are binding sites on DNA and are not transcribed.

E. coli has numerous ways to control the transcription of operons, and we will focus on three of them. Two ways depend on interactions of the repressor protein with the operator, and the third depends on interactions of other proteins with the promoter. Let's consider each of these three control systems in turn.

Operator-repressor control that induces transcription: The lac operon

The operon that controls and contains the structural genes for the three /tftose-metabolizing enzymes is called the lac operon (see Figure 13.16). As we have just learned, RNA polymerase can bind to the promoter, and a repressor can bind to the operator.

How is the operon controlled? The key lies in the repressor and its binding to the operator. The repressor protein has two binding sites: one for the operator and the other for inducers. The inducers of the lac operon, as we know, are molecules of lactose and certain other (3-galactosides. Binding of an inducer changes the shape of the repressor (by al-losteric modification; see Chapter 6). This change in shape prevents the repressor from binding to the operator (Figure 13.17). As a result, RNA polymerase can bind to the promoter and start transcribing the structural genes of the lac operon. The mRNA transcribed from these genes is translated on ribosomes, synthesizing the three proteins required for metabolizing lactose.

What happens if the concentration of lactose drops? As the lactose concentration decreases, the inducer (lactose) molecules separate from the repressor. Free of lactose molecules, the repressor returns to its original shape and binds to the operator, and transcription of the lac operon stops. Translation stops soon thereafter, because the mRNA that is already present breaks down quickly. Thus, it is the pres-

13.16 The lac Operon of E. coli and Its Regulator

The lac operon of E. coli is a segment of DNA that includes a promoter, an operator, and the three structural genes that code for the lactose-metabolizing enzymes.

Operon

f

...structural genes, such as metabolic enzymes.

V

Structural genes-

DNA

Promoter for Regulatory gene Promoter for Operator regulatory gene /{ structural genes

HA regu

latory gene codes for a repressor protein that binds the operator to turn off structural genes.

Structural gene for P-galactosidase

Structural gene for (3-galactoside permease

Structural gene for f3-galactoside transacetylase

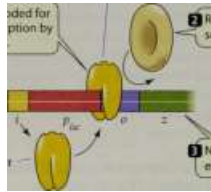
Lactose absent

Q The repressor protein coded for gene / prevents transcription by binding to the operator.

Repressor bound to operator

DNA

Active repressor



RNA polymerase cannot bind, so transcription is blocked.



No mRNA is produced, so no enzyme is produced.

Lactose present

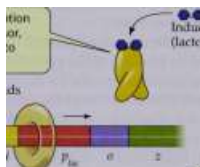
Q Lactose induces transcription by binding to the repressor, which cannot then bind to the operator.

RNA polymerase binds promoter

Inducer

(lactose)

DNA



Transcription proceeds

H As long as the operator

remains free of repressor, RNA I polymerase can transcribe the /xi genes for enzymes.

DNA



mRNA transcript is produced.

ence or absence of lactose—the inducer—that regulates the binding of the repressor to the operator, and therefore the synthesis of the proteins needed to metabolize it.

Repressor proteins are coded by regulatory genes. The regulatory gene that codes for the repressor of the lac operon is called the *i* (mducibility) gene. The *i* gene happens to lie close to the operon that it controls, but some other regulatory genes are distant from their operons. Like all other genes, the *i* gene itself has a promoter, which can be designated *p_t* . Because this promoter does not bind RNA polymerase very effectively, only enough mRNA to synthesize about ten molecules of repressor protein per cell per generation is produced. This quantity of the repressor is enough to regulate the operon effectively—to produce more would be a waste of energy. There is no operator between *p_t* and the *i* gene. Therefore, the repressor of the lac operon is constitutive; that is, it is made at a constant rate that is not subject to environmental control.

Let's review the important features of inducible systems such as the lac operon:

► In the absence of inducer, the lac operon is turned off.

13.17 The lac Operon: Transcription Is Induced by the Removal of a Repressor

Lactose (the inducer) leads to enzyme synthesis by preventing the repressor protein (which would have stopped transcription) from binding to the operator.

Control is exerted by a regulatory protein—the repressor—that turns the operon off.

Some genes, such as *i*, produce proteins whose sole function is to regulate the expression of other genes. Certain other DNA sequences (operators and promoters) do not code for proteins but are binding sites for regulatory or other proteins.

Adding inducer turns the operon on.

Operator-repressor control that represses transcription: The *trp* operon

We have seen that *E. coli* benefits from having an inducible system for lactose metabolism. Only when lactose is present does the system switch on. Equally valuable to a bacterium is the ability to switch off the synthesis of certain enzymes in response to the excessive accumulation of their end products. For example, if the amino acid tryptophan, an essential constituent of proteins, is present in ample concentration, it is advantageous to stop making the enzymes for tryptophan synthesis. When the synthesis of an enzyme can be turned off in response to such a biochemical cue, it is said to be repressible.

The French biochemist Jacques Monod, who had been part of the team that deciphered the *lac* operon, realized that repressible systems, such as the *trp* operon for tryptophan synthesis, could work by mechanisms similar to those of inducible systems. In repressible systems, the repressor protein cannot shut off its operon unless it first binds to a corepressor, which may be either the metabolite itself (tryptophan in this case) or an analog of it (Figure 13.18). If the metabolite is absent, the operon is transcribed at a maximum rate. If the metabolite is present, the operon is turned off.

The difference between inducible and repressible systems is small, but significant. In inducible systems, a substance in the environment (the inducer) interacts with the regulatory gene product (the repressor), rendering it incapable of binding to the operator and thus incapable of blocking transcription. In repressible systems, a substance in the cell (the corepressor) interacts with the regulatory gene product to make it capable of binding to the operator and

Tryptophan absent

DNA

THE GENETICS OF VIRUSES AND PROKARYOTES 253

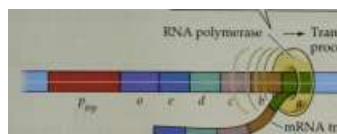
mRNA

Inactive repressor



Regulatory gene *trpR* produces an inactive repressor, which cannot bind to the operator.

RNA polymerase transcribes the structural genes. Translation makes the enzymes of the tryptophan pathway.



Transcription proceeds

DNA

mRNA transcript

Enzymes of the

tryptophan pathway

IIII

Translation

£3 »

b a

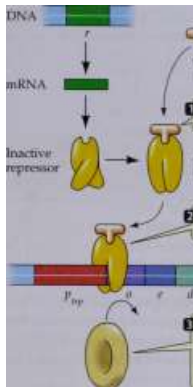
13.18 The *trp* Operon: Transcription Is Repressed by the Binding of a Repressor

Because tryptophan activates an otherwise inactive repressor, it is called a corepressor.



Tryptophan present DNA

mRNA



5P

Corepressor (tryptophan)

Tryptophan binds the repressor...

Active repressor

...which then binds to the operator.

DNA

Tryptophan blocks RNA polymerase from binding and transcribing the structural genes, preventing synthesis of tryptophan pathway enzymes.

blocking transcription. Although the effects of the substances are exactly opposite, the systems as a whole are strikingly similar.

In both the inducible lactose system and the repressible tryptophan system, the regulatory molecule functions by binding the operator. Next we'll consider an example of control by binding the promoter.

Protein synthesis can be controlled by increasing promoter efficiency

The mechanisms of transcriptional regulation that we have discussed thus far involve repressor-operator interactions that turn the operon on or off. Another way to regulate transcription is to make the promoter work more efficiently.

Suppose that a bacterial cell lacks a supply of glucose, its preferred energy source, but instead has access to another sugar (e.g., lactose or maltose) that can be broken down to enter an energy pathway. In operons such as the lac operon that have genes for enzymes that catabolize such alternative energy sources, the promoters bind RNA polymerase in a series of steps (Figure 13.19). First, a protein called CRP (short for

f

When glucose levels are low...

<

Low glucose

CRP-cAMP

complex

(enhancer)

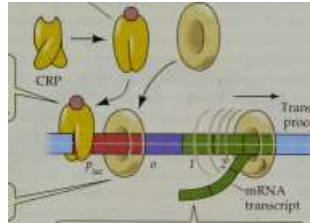
1

cAMP

Q ...a receptor protein (CRP) and cAMP complex binds to the promoter, activating it.

f

RNA polymerase then binds the promoter...



Transcription proceeds

DNA

f

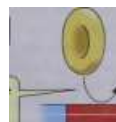
...and transcribes genes encoding enzymes that catabolize an alternative energy source.

t

When glucose levels are high.

High glucose

...RNA polymerase cannot bind efficiently.



~ir ii

13.19 Transcription Is Enhanced by the Binding of the CRP-cAMP Complex to the Promoter

The structural genes of this operon encode enzymes that break down a food source other than glucose.

'toe

t

k

Structural genes are not transcribed. This is adaptive when the cell does not require an alternative energy source.

254 CHAPTER THIRTEEN

13.2

The Relationships Between Positive and Negative Control in the lac Operon

GLUCOSE

cAMP LEVELS

RNA POLYMERASE BINDING TO PROMOTER

LACTOSE

LAC REPRESSOR

TRANSCRIPTION OF LAC GENES?

LACTOSE USED BY CELLS?

cAMP receptor protein) binds the low-molecular-weight compound adenosine 3',5'-cyclic monophosphate, better known as cyclic AMP, or cAMP. Next, the CRP-cAMP complex binds to DNA just upstream of the promoter. This binding results in more efficient binding of RNA polymerase to the promoter, and an elevated level of transcription of the structural genes.

When glucose becomes abundant in the medium, the bacteria do not need to break down alternative food molecules, so the cell diminishes or ceases synthesizing the enzymes that catabolize these alternative sources. Glucose decreases the synthesis of these enzymes—a phenomenon called catabolite repression —by lowering the cellular concentration of cAMP.

As you will see in later chapters of this book, cAMP is a widely used signaling molecule in eukaryotes, as well as in prokaryotes. The use of this nucleotide in such widely diverse situations as a bacterium sensing glucose levels and humans

sensing hunger demonstrates the prevalence of common themes in biochemistry and natural selection.

The lac and trp systems—the two operator-repressor systems—are examples of negative control of transcription because the regulatory molecule (the repressor) in each case prevents transcription. The promoter system is an example of positive control of transcription because the regulatory molecule (the CRP-cAMP complex) enhances transcription. The relationships between these positive and negative systems are summarized in Table 13.2.

Control of Transcription in Viruses

The mechanisms used by prokaryotes for the regulation of gene expression are also used by viruses. Even a "simple" biological agent such as a virus is faced with complicated molecular decisions when its genome enters a cell. For example, the viral genome must direct the shutdown of host transcription and translation, then redirect the host protein synthesis machinery to virus production. Genes must be activated in the right order; it makes little sense, for example, for the viral genome to transcribe and translate proteins that lyse the host cell membrane before the virus particles are assembled, ready for release. In temperate viruses,

which insert their genome (or a DNA copy) into the host chromosome, another issue arises: When should the pro-virus leave the host chromosome and undertake a lytic cycle?

Bacteriophage lambda is a temperate phage, which can undergo either a lytic or a lysogenic cycle (see Figure 13.2). When there is a rich medium and its host bacteria are growing, the phage takes advantage of its favorable environment (lots of resources for the phage in the host cell cytoplasm) and undergoes a lytic cycle. When the host bacteria are not as healthy, the phage senses this, and "lays low" as a lysogenic prophage. When things improve, the prophage leaves the host chromosome and becomes lytic.

The phage makes this decision by means of a "genetic switch": Two regulatory proteins compete for two operator/promoter sites on phage DNA. The two operators control the transcription of genes involved in the lytic and the lysogenic cycles, respectively, and the two regulatory proteins have opposite effects on the two operators (Figure 13.20):

PROTEIN

LYTIC OPERATOR/ PROMOTER

LYSOGENIC OPERATOR/PROMOTER

Cl

Cro

Represses Activates

Activates Represses

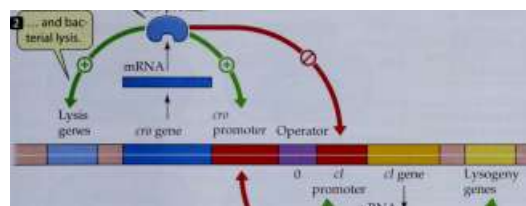
So phage infection is a "race" between these two regulatory proteins. If cl "wins," which occurs when Cro synthesis is low in an unhealthy *E. coli* host cell, the phage enters a lysogenic cycle. If the host cell is healthy, a lot of Cro is made, lysogeny is blocked, and lysis ensues. These regulatory proteins, made very early in phage infection, both have binding domains for recognition of specific phage DNA sequences.

The life cycle of phage lambda, which has been greatly simplified here, is a paradigm for viral infections throughout the biological world. The lessons learned from transcriptional controls in this system have been applied again and again to other viruses, including HIV. The control of gene activity in eukaryotic cells is somewhat different, as we will see in the next chapter, but nevertheless usually involves protein-DNA interactions.

Q When the bacterial host is healthy, Cro

protein accumulates, activates promoters for phage DNA replication, coat proteins.,

^\Cro protein



\

cl

promoter

mRNA

Lysogeny genes

i

f

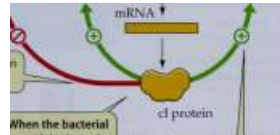
cl protein breaks down when bacterial growth rapid.

73.20 Control of Phage Lambda Lysis and Lysogeny

Two regulatory proteins, Cro and

cl, compete for the operator/promoters

controlling lysis and lysogeny.



O When the bacterial growth is slow, cl

accumulates...

Prokaryotic Genomes

When DNA sequencing first became possible in the late 1970s, the first biological agents to be sequenced were the simplest viruses.

Soon, over 150 viral genomes, including those of important animal and plant pathogens, were sequenced. Information on how they infected and reproduced came quickly as a result.

But manual sequencing was not up to the task of elucidating the genomes of prokaryotes and eukaryotes, the smallest of which are 100 times larger than those of a bacteriophage. In the past 6 years, however, the automation of sequencing has rapidly added many prokaryotic sequences to the biologists' store of knowledge.

In 1995, a team led by Craig Venter and Hamilton Smith determined the first sequence of a free-living organism, the bacterium *Haemophilus influenzae*. Many more sequences have followed, and they have revealed not only how these organisms apportion their genes to perform different cellular roles, but how their specialized functions are carried out. A beginning has even been made on the provocative question of what the minimal requirements for a living cell might be.

Three types of information can be obtained from a genomic sequence:

- Open reading frames, which are the coding regions of genes. For protein-coding genes, these regions can be recognized by the start and stop codons for translation.
- Amino acid sequences. For proteins, these can be deduced from the DNA sequence by looking up the genetic code.
- Gene control sequences, such as promoters and terminators for transcription.

THE GENETICS OF VIRUSES AND PROKARYOTES 255

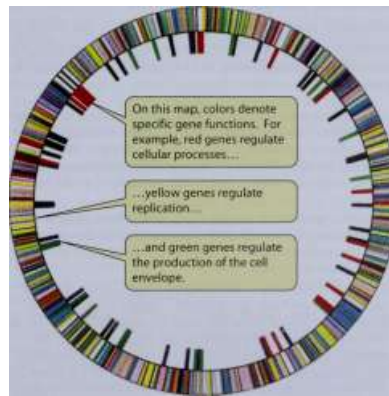
Functional genomics relates gene sequences to functions

The only host for the bacterium *Haemophilus influenzae* is humans. It lives in the upper respiratory tract and can cause ear infections or, more seriously, meningitis in children. Its 1,830,137 base pairs are in a single circular chromosome (Figure 13.21). In addition to its origin of DNA replication and genes coding for rRNA's and tRNA's, this bacterial chromosome has 1,743 regions containing amino acid codons along with the transcriptional (promoter) and translational (start and stop codons) information needed for protein synthesis.

When this sequence was announced, only 1,007 (58%) of its genes had amino acid sequences that corresponded to proteins with known functions—in other words, were genes that researchers, based on their knowledge of the functions of bacteria, expected to find. Roles for most of the unknown proteins have been identified since then, a process known as annotation. Functional genomics, the assignment of roles to genes and the description of how they work in the organism, has become the major occupation of many biologists.

Of the genes and proteins with known roles, most confirm a century of biochemical description of bacterial enzymatic pathways. For example, there are genes for the entire pathways of glycolysis, fermentation, and electron transport.

o ...and activates promoters for integration of phage DNA into the host chromosome.



73.2 7 Functional Organization of the Genome of *H. influenzae*

The entire DNA sequence has 1,830,137 base pairs.

256 CHAPTER THIRTEEN

METHOD

M. genitalium has 470 genes; only two are shown here.

A transposon inserts randomly into one gene...

...inactivating it.

The mutated bacterium is put into growth medium.

RESULTS

Some of the gene sequences for unknown proteins may code for membrane proteins, possibly those involved in active transport. Another important finding is that highly infective strains of this bacterium have genes coding for surface proteins that attach them to the human respiratory tract, while noninfective strains lack those genes.

Soon after the sequence of *H. influenzae* was announced, smaller (*Mycoplasma genitalium*, 580,070 base pairs) and larger (*E. coli*, 4,639,221 base pairs) prokaryotic sequences were completed. Thus began a new era in biology, the era of comparative genomics, in which genome sequences are compared to see what genes one organism has or is missing, in order to relate the results to physiology.

M. genitalium, for example, lacks the enzymes needed to synthesize amino acids, which the other two organisms possess. This finding reveals that *M. genitalium* is a parasite, which must obtain all its amino acids from its environment, the human urogenital tract. *E. coli* has 55 genes coding for transcriptional activation and 58 repressors; *M. genitalium* has only 3 activators. Comparisons such as these have led to the formulation of specific questions about how an organism lives the way it does.

The sequencing of prokaryotic genomes has medical applications

Sequencing has important ramifications for the study of prokaryotes that cause human diseases. Indeed, most of the early efforts in sequencing have focused on human pathogens.

► *Chlamydia trachomatis* causes the most common sexually transmitted disease in the United States. Because it is an intracellular parasite, it has been very hard to study. Among its 900 genes are ones for ATP synthesis—something scientists used to think this bacterium could not do:

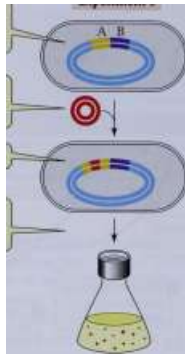
► *Rickettsia prowazekii* causes typhus; it infects people bitten by lice vectors. Of its 634 genes, 6 code for proteins that are essential for its virulence. These genes are being used to develop vaccines.

► *Mycobacterium tuberculosis* causes tuberculosis. It has a large (for a prokaryote) genome, coding for 4,000 proteins. Over 250 of these are used to metabolize lipids, so this may be the main way that the bacterium gets its energy. Some genes coding for previously unidentified cell surface proteins are targets for potential vaccines. The sequence has enough similarities to those of mitochondria to lead to the proposal that this bacterium's ancestor was the one that colonized a cell to ultimately produce that organelle.

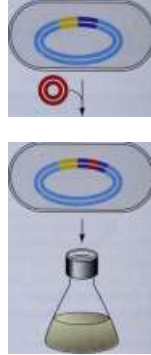
EXPERIMENT

Question: Are all genes in a genome essential for cell survival? Can transposon mutagenesis be used to determine which genes are essential for cell survival?

Experiment 1



Experiment 2



Growth means that gene A is not essential.

No growth means that gene B is essential.

Conclusion: If each gene is inactivated in turn by mutation, a "minimal essential genome" can be determined.

13.22 Using Transposon Mutagenesis to Determine the Minimal Genome

By inactivating genes one by one, scientists can determine which ones are essential for the cell's survival.

Sequencing has also provided the necessary information for the design of primers and hybridization probes used to detect these and other pathogens.

What genes are required for cellular life?

One striking conclusion arising from comparing the genomes of prokaryotes and eukaryotes is that there are some truly universal genes, present in all organisms. There are also some universal gene segments—coding for an ATP binding site, for example—that are present in many genes in many organisms. These findings suggest that there is some ancient, minimal set of DNA sequences that all cells must have. One way to identify these sequences is to look for them (or, more realistically, to have a computer look for them).

Another way to define a minimal genome is to take the simplest genome and deliberately mutate one gene at a time and see what happens. *M. genitalium*, with only 470 genes, has the smallest known genome of any organism. Some of its genes are dispensable under some circumstances: There are genes for using both glucose and fructose, and in the laboratory, the organism could survive on only one of those sugars, making the genes for the other unnecessary. But what about other genes? Experiments

THE GENETICS OF VIRUSES AND PROKARYOTES 257

using transposons as mutagens have been performed to address this question. The transposons insert themselves into a gene at random, mutating and inactivating it (Figure 13.22). The resulting mutated cell is sequenced to determine which gene was mutated, and then examined for growth and survival.

The astonishing result of these studies is that *M. genitalium* can survive in the laboratory without the services of 133 of its genes, leaving a minimum of 337 genes! Putting it another way, these 337 genes could theoretically be spliced together (or even synthesized in the laboratory) to make totally artificial life. It is not surprising that the scientists involved in this research have convened a panel of theologians, philosophers and lawyers to advise them.



Chapter Summary

Using Prokaryotes and Viruses to Probe the Nature of Genes

► Prokaryotes and viruses are useful for the study of genetics and molecular biology because they contain much less DNA

than eukaryotes, they grow and reproduce rapidly, and they are haploid.

Viruses: Reproduction and Recombination

- ▶ Viruses were discovered as disease-causing agents small enough to pass through a filter that retains bacteria. They consist of a nucleic acid genome, which codes for a few proteins, and a protein coat. Some viruses also have a lipid membrane derived from host membranes.
- ▶ Viruses are obligate intracellular parasites, needing the biochemical machinery of living cells to reproduce.
- ▶ There are many types of viruses, classified by their size and shape, by their genetic material (RNA or DNA), or by their host organism. Review Figure 13.1
- ▶ Bacteriophages are viruses that infect bacteria. In the lytic cycle, the host cell breaks open, releasing many new phage particles. Some phages can also undergo a lysogenic cycle, in which their DNA is inserted into the host chromosome, where it replicates for generations. When conditions are appropriate, the lysogenic DNA exits the host chromosome and enters a lytic cycle. Review Figure 13.2
- ▶ Some viruses have promoters for host RNA polymerase, which they use to transcribe their own genes. Review Figure 13.3
- ▶ Most of the many types of RNA and DNA viruses that infect animals cause diseases. Some animal viruses are surrounded by membranes derived from the host's plasma membrane. Retroviruses, such as HIV, have RNA genomes that they reproduce through a DNA intermediate. Other RNA viruses use their RNA as mRNA to code for enzymes and replicate their genomes without using DNA. Review Figures 13.4, 13.5, and Table 13.2
- ▶ Many plant viruses are spread by other organisms, such as insects.
- ▶ Viroids are made only of RNA and infect plants, where they are replicated by the plant's enzymes.

Prokaryotes: Reproduction, Mutation, and Recombination

- ▶ When bacteria divide, they form clones of identical cells that can be observed as colonies when grown on solid media. Review Figure 13.6
- ▶ A bacterium can transfer its genes to another bacterium by conjugation, transformation, or transduction.
- ▶ In conjugation, a bacterium attaches to another bacterium and passes a partial copy of its DNA to the adjacent cell. Review Figures 13.7, 13.8, 13.9
- ▶ In transformation, genes are transferred between cells when fragments of bacterial DNA are taken up by a cell from the medium. These genetic fragments may recombine with the host chromosome, thereby permanently adding new genes. Review Figure 13.10
- ▶ In transduction, phage capsids carry bacterial DNA from one bacterium to another. Review Figure 13.10
- ▶ Plasmids are small bacterial chromosomes that are independent of the main chromosome. R factors, which are plasmids that carry genes for antibiotic resistance, are a serious public health threat. Review Figure 13.11
- ▶ Transposable elements are movable stretches of DNA that can jump from one place to another on the bacterial chromosome—either by actually moving or by making a new copy, which is inserted at a new location. Review Figure 13.12

Regulation of Gene Expression in Prokaryotes

- ▶ In prokaryotes, the expression of some genes is regulated; their products are made only when they are needed. Other genes, called constitutive genes, whose products are essential to the cell at all times, are constantly expressed. A compound that stimulates the synthesis of an enzyme needed to process it is called an inducer. Review Figures 13.13, 13.14
- ▶ An operon consists of a promoter, an operator, and a number of structural genes. Promoters and operators do not code for proteins, but serve as binding sites for regulatory proteins. When a repressor protein binds to the operator, transcription of the structural genes is inhibited. Review Figures 13.15, 13.16
- ▶ The expression of prokaryotic genes is regulated by three different mechanisms: inducible operator-repressor systems, repressible operator-repressor systems, and systems that increase the efficiency of a promoter.
- ▶ The lac operon is an example of an inducible system whose proteins allow bacteria to metabolize lactose. When lactose is absent, a repressor protein binds tightly to the operator. The repressor prevents RNA polymerase from binding to the promoter, turning transcription off. When glucose is absent and lactose is present, lactose acts as an inducer by binding to the repressor. This changes the repressor's shape so that it no longer recognizes the operator. With the operator unbound, RNA polymerase binds to the promoter, and transcription is turned on.
- ▶ Repressor proteins are coded by constitutive regulatory genes.
- ▶ The trp operon is a repressible system in which the presence of the end product of a biochemical pathway, tryptophan, represses the synthesis of enzymes involved in its own synthesis. Tryptophan acts as a corepressor by binding to an inactive

repressor protein and making it active. When the activated repressor binds to the operator, transcription is turned off. Review Figure 13.18

► The efficiency of RNA polymerase can be increased by regulation of the level of cyclic AMP, which binds to a protein called CRP. The CRP-cAMP complex then binds to a site near the promoter of a target gene, enhancing the binding of RNA polymerase and hence transcription. Review Figure 13.19

Control of Transcription in Viruses

- In bacteriophages that can undergo a lytic or a lysogenic cycle, the decision as to which pathway to take is made by

258 CHAPTER THIRTEEN

operator-regulatory protein interactions. Review Figure 13.20

Prokaryotic Genomes

- Functional genomics relates gene sequences to functions. Review Figure 13.21
- By mutating individual genes in a small genome, scientists can determine the minimal genome required for a prokaryote. Review Figure 13.22

For Discussion

1. Viruses sometimes carry DNA from one cell to another by transduction. Sometimes a segment of bacterial DNA is incorporated into a phage capsid without any phage DNA. These particles can infect a new host. Would the new host become lysogenic if the phage originally came from a lysogenic host? Why or why not?
2. Compare the life cycles of the viruses that cause influenza (Figure 13.4) and AIDS (Figure 13.5) with respect to how the virus enters the cell; how the virion is released into the cell; how the viral genome is replicated; and how new virus particles are produced.
- 6.

Lederberg, Tatum, and colleagues were able to rule out new mutation and transformation as explanations for the prototrophic colonies that appeared when they mixed cultures of different auxotrophic *E. coli*. Propose experiments to rule out each of these alternatives.

Compare promoters adjacent to "early" and "late" genes in the viral life cycle.

The repressor protein that turns off the lac operon of *E. coli* is encoded by a regulatory gene. The repressor molecules are made in very small quantities and at a constant rate per cell. Would you surmise that the promoter for these repressor molecules is efficient or inefficient? Is synthesis of the repressor constitutive, or is it under environmental control?

A key characteristic of a repressible enzyme system is that the repressor molecule must react with a corepressor (typically, the end product of a metabolic pathway) before it can combine with the operator of an operon to shut the operon off. How is this different from an inducible enzyme system?



The Eukaryotic Genome and Its Expression

- When Tom was diagnosed with leukemia—cancer of the blood cells—his initial treatment included chemotherapy. Combinations of powerful antimitotic drugs were administered to kill the rapidly dividing cancer cells that were spreading throughout his body. But the dosages his physicians prescribed were not up to the task, and the cells continued to spread. Higher dosages of these chemotherapeutic drugs would be lethal; they would kill not only the cancer cells, but the healthy and essential cells in the bone marrow that divide by the hundreds of millions to form blood cells. Without these cells, Tom's bone marrow would no longer produce red blood cells with their vital oxygen-carrying protein hemoglobin, nor would he be able to produce white blood cells, which make the proteins that combat infectious diseases as well as some tumors.

Tom's doctors tried a new approach. They extracted some of his bone marrow and removed the cancer cells from it, then stored the marrow in a refrigerator. Then they gave Tom extremely high doses of the chemotherapeutic drugs, which killed the cancer cells. Finally, the stored bone marrow was replaced in Tom's body. The healthy bone marrow cells began to divide, and after a few weeks they were forming populations of normal red and white blood cells. Tom's leukemia had disappeared.

The success of Tom's bone marrow transplant depended on many things, but the principle behind it is based on the specificity of gene expression during cell differentiation. What are the genetic mechanisms that ensure that healthy red blood cells will contain hemoglobin, and that white blood cells are able to create the vital antibody proteins of the immune system? What features of the DNA sequences of eukaryotic genes determine these mechanisms, and how do they differ from the genes that code for proteins in prokaryotes?

In this chapter, you will see that, although both prokaryotes and eukaryotes use DNA as genetic material, eukaryotic DNA differs from prokaryotic DNA in both its content and its

Two Cells, Two Different Protein Products

The red blood cells—erythrocytes—contain abundant hemoglobin, while the white blood cells synthesize proteins of the immune system.

organization. In addition to the genes for metabolism that prokaryotes have, eukaryotes have genes that mark them as complex cells: genes for addressing, or targeting, proteins to organelles, and genes for cell-cell interaction and cell differentiation.

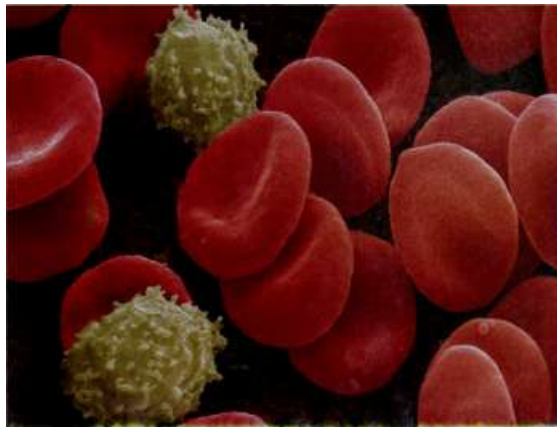
Unlike prokaryotes, eukaryotes have repetitive sequences of DNA, many of which do not code for proteins. In addition, the transcription and later tailoring of mRNA is more complicated in eukaryotes than in prokaryotes. Elegant molecular machinery allows the precise regulation of gene expression needed for all the cells of these complex organisms to develop and function.

The Eukaryotic Genome

As biologists unraveled the intricacies of gene structure and expression in prokaryotes, they tried to generalize their findings by saying, "What's true for *E. coli* is also true for elephants." Although much of prokaryotic biochemistry does apply to eukaryotes, the old saying has its limitations. Table 14.1 lists some of the differences between prokaryotic and eukaryotic genomes.

The eukaryotic genome is larger and more complex than the prokaryotic genome

The fact that the genome of eukaryotes (in terms of haploid DNA content) is larger than that of prokaryotes might be



260 CHAPTER FOURTEEN

expected, given that in multicellular organisms there are many cell types, many jobs to do, and many proteins—all coded for by DNA—to do those jobs. A typical virus contains only enough DNA to code for a few proteins—about 10,000 base pairs (bp). The most thoroughly studied pro-karyote, *E. coli*, has sufficient DNA (about 4.5 million bp) to make several thousand different proteins and regulate their synthesis. Humans have considerably more genes and regulators: Nearly 6 billion bp (2 meters of DNA) are crammed into each human cell. However, the idea of a more complex organism needing more DNA seems to break down with some plants. For example, the lily (which produces beautiful flowers each spring, but produces fewer proteins than a human does) has 18 times more DNA than humans have (Figure 14.1).

As we will see, the organization of the nuclear eukaryotic genome is fundamentally about regulation. The great complexity of eukaryotes requires a great deal of regulation, and this fact is evident in the many processes and points of control associated with the expression of the eukaryotic genome.

Unlike prokaryotic DNA, most eukaryotic DNA is noncoding. Interspersed throughout the eukaryotic genome are various kinds of repeated DNA sequences that are not transcribed into proteins. Even the coding regions of genes contain sequences that do not end up in mature mRNA.

Some of this noncoding DNA maintains structural integrity at the ends of chromosomes, and some regulates gene expression. But the presence of much of this non-coding DNA remains an enigma.



Bacteriophage (virus) 10,000 bp

In contrast to the single main chromosome of most prokaryotes, the eukaryotic genome is partitioned into several separate chromosomes. In humans, each chromosome contains a double helix of DNA with 20 million to 100 million bp. This separation of genomic encyclopedia into multiple volumes requires that each chromosome have, at a minimum, three defining DNA sequences: Recognition sequences for the DNA replication machinery, a centromere region that holds the replicated sequences together before mitosis, and a telomeric sequence at each end of the chromosome. We have described the roles of the first two types of sequences in previous chapters, and will discuss telomeres later in this chapter.

In eukaryotes, the nuclear envelope separates DNA and its transcription (inside the nucleus) from the cytoplasmic sites where mRNA is translated into protein. This separation allows for many points of control in the synthesis, processing, and transport of mRNA to the cytoplasm.



We expect simple organisms to have small genomes...

Yeast

24 million bp

E. coli

4 million bp

Caenorhabditis elegans

(roundworm)

160 million bp per cell

Fruit fly

330 million bp

per cell

Lily

106 billion bp

per cell

__A

14.1 Amounts of Genomic DNA Can Be Deceiving

Eukaryotes have more DNA in their genomes than prokaryotes. However, among some eukaryotes—especially plants—there is no apparent relationship between diploid genome size and organism complexity.

...but why does a lily have 18 times the DNA that a human does?



Human 6 billion bp per cell

IDNA in the nucleus contains genes that code for specific proteins.

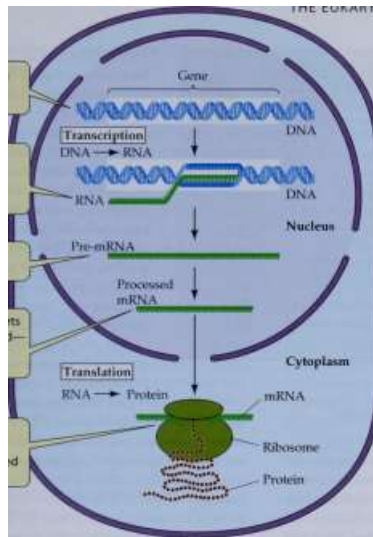
Under the right conditions, these genes are transcribed to make messenger RNA (see Chapter 12).

f

Pre-mRNA transcript is produced.

I Pre-mRNA is processed—parts are removed, ends are added-and the resulting mRNA is exported to the cytoplasm.

In the cytoplasm, ribosomes translate the mRNA to make a protein (polypeptide) coded by the gene.



THE EUKARYOTIC GENOME AND ITS EXPRESSION 261

rn



74.2 Eukaryotic mRNA Is Processed in the Nucleus and Exported to the Cytoplasm

Compare this "road map" to the prokaryotic one shown in Figure 12.3.

Most eukaryotic DNA is not even fully exposed to the nuclear environment. Instead, it is extensively packaged by proteins into nucleosomes, chromatin fibers, and ultimately chromosomes (see Figure 9.7). This extensive compaction is a means of restricting access of the RNA synthesis machinery to the DNA, as well as a way of segregating replicated DNA's during mitosis and meiosis.

Like the genes of prokaryotes that code for proteins, eukaryotic genes are flanked by noncoding sequences that regulate their transcription. These include the promoter region, where RNA polymerase binds to begin transcription. Of equal importance in eukaryotes, but rare in prokaryotes, is a second set of regulatory DNA sequences, the enhancers and silencers. These sequences are often located quite far from the promoter, and they act by binding proteins that then stimulate or inhibit transcription.

The noncoding DNA sequences found within protein-coding genes present a special problem: How do cells ensure that transcripts of these noncoding regions do not end up in the mRNA that exits the nucleus? The answer lies in an elaborate cutting and splicing mechanism within the nucleus that modifies the initial transcript, called pre-mRNA, by cutting out the noncoding regions after transcription (Figure 14.2). Thus, in contrast to the "what is transcribed is what is translated" scheme of most prokaryotic genes, the mature mRNA that is translated at the eukaryotic ribosome is a modified and much smaller molecule than the one initially made in the nucleus.

As we described in Chapter 13, advances in the automation of DNA sequencing have made it possible to obtain the

entire sequences of many prokaryotic genomes. A next step in size and complexity is the sequencing of simple eukaryotes. This has been achieved for a single-celled organism, yeast, as well as for the multicellular roundworm *Caenorhabditis elegans*. Further breakthroughs in the speed and sophistication of the equipment used to sequence DNA accelerated the work on complex eukaryotic genomes, and complete base sequencing of the fruit fly *Drosophila melanogaster* was completed in late 1999. And summer of the year 2000 saw the announcement, attended by frenzied media coverage, of the complete base sequencing of the human genome.

The yeast genome adds some eukaryotic functions onto a prokaryotic model

In comparison with *E. coli*, whose genome has about 4,500,000 bp on a single chromosome, the genome of budding yeast, *Saccharomyces cerevisiae*, has 16 chromosomes and a haploid content of more than 12,068,000 bp. More than 600 scientists around the world collaborated in mapping and sequencing the yeast genome. When they began, they knew of about 1,000 yeast genes coding for RNA or protein. The final sequence revealed 6,200 genes; sequence analyses have assigned probable roles to about 70 percent of them. The functions of the other 30 percent are being investigated by gene inactivation studies similar to those performed on prokaryotes (see Figure 13.22).

It is now possible to estimate what proportions of the yeast genome code for specific metabolic roles. Apparently, 11 percent of yeast proteins are for general metabolism, 3 percent for energy production and storage, 3 percent for DNA replication and repair, 12 percent for protein synthesis, and 6 percent for protein targeting and secretion. Many of the others are involved in cell structure, cell division, and the regulation of gene expression.

The most striking difference between the yeast genome and that of *E. coli* is in the genes for protein targeting (Table 14.2). Both of these single-celled organisms appear to use about the same number of genes to perform the basic functions of cell survival. It is the compartmentalization of the eukaryotic cell into organelles that requires it to have many more genes. This

finding is direct, quantitative confirmation of something we have known for a century: The eukaryotic cell is structurally more complex than the prokaryotic cell.

262 CHAPTER FOURTEEN

The nematode genome adds developmental complexity

The presence of more than a single cell adds a new level of complexity to the genome. *Caenorhabditis elegans* is a 1-mm long nematode (roundworm) that normally lives in the soil. But it also lives in the laboratory, where it is a favorite organism of developmental biologists (see Chapter 16). It has a transparent body, through which scientists can watch over 3 days as its fertilized egg divides and forms an adult worm of 1,000 cells. In spite of its small number of cells, the adult worm has a nervous system, digests food, reproduces sexually, and ages. So it is not surprising that an intense effort was made to sequence the genome of this organism.

Just as with yeast and *E. coli*, the computer-based science of comparative genomics has given us much information on the *C. elegans* genome. It is eight times larger than that of yeast (97 million base pairs) and has four times more protein-coding genes (19,099). Once again, sequencing revealed far more than expected: When the sequencing effort began, researchers estimated that the worm would have about 6,000 proteins.

About 3,000 genes in the worm have direct homologs in yeast; these genes code for basic eukaryotic cell functions. What do the rest of the genes—the bulk of the worm genome—do? In addition to surviving, growing, and dividing, as single-celled organisms do, multicellular organisms must have genes for holding cells together to form tissues, for cell differentiation to divide up tasks in the organism, and for intercellular communication to coordinate its activities (Table 14.3). Many of the genes so far identified in *C. elegans* that are not present in yeast perform these roles, which will be described in the remainder of this chapter and the next one.

The fruit fly genome has surprisingly few genes

The fruit fly *Drosophila melanogaster* is a much larger organism than *C. elegans*, both in size (the fly has 10 times more cells) and complexity. Not surprisingly, the fly genome is

also larger, about 180,000,000 base pairs. New technologies made it possible to sequence the entire *Drosophila* genome in about a year.

Even before the complete sequence was announced, decades of genetic studies had already identified some 2,500 different genes in the fly. These genes were all found in the complete DNA sequence, along with many other genes whose functions are as yet unidentified. Efforts are now under way to determine what these genes do in the life of the fly. (This process of discovering the protein product and function of a known gene sequence is called annotation.)

But the big surprise of the *Drosophila* genome sequence was the total number of protein-coding regions. Instead of being higher than the roundworm's (18,000 genes), the fly has only 13,600 genes. One reason for this is that the roundworm has some large gene families, which, as we will see later in this chapter, are groups of genes related in their sequence and function. For example, *C. elegans* has 1,100 genes involved in either nerve cell signaling or development; a fly has only 160 genes for these two functions. Another major expansion in the worm is in its genes coding for proteins that sense chemicals in its environment.

Many genes that are present in the worm genome have homologs with similar sequences in fly DNA, accounting for a third of the fly genes. And about half of the fly genes have mammalian homologs. An important medical contribution of comparative genomics has resulted from finding genes that are implicated in human diseases in other organisms. Often the roles of such genes can be elucidated in the simpler organism, providing a clue to how the gene might function in human disease. The fly genome contains 177 genes whose sequences are known to be directly involved in human diseases, including cancer and neurological conditions.

Gene sequences for other organisms are rapidly becoming known

A "rough" human genome sequence is already available, with a more detailed one just a few years away. The human genome sequence and its myriad implications will be discussed in Chapter 18. Meanwhile, sequencing is proceeding rapidly for another model organism: the weedy plant *Arabidopsis thaliana* (130 million base pairs). These eukaryotic sequences will pose great challenges and opportunities for biologists in the next decades. "The sequence is not the end of the day," says Sydney Brenner, a leader of this effort. "It's the beginning of the day."

Repetitive Sequences in the Eukaryotic Genome

As we have mentioned, and as you have seen in the genome sequences we have examined, the eukaryotic genome has some base sequences that are repeated many times. Some of these sequences are present in millions of copies in a single genome. In this section, we will examine the organization and possible roles of these repetitive sequences.

Highly repetitive sequences are present in large numbers of copies

Three types of highly repetitive sequences are found in eukaryotes:

► Satellites are 5-50 base pairs long, repeated side by side up to a million times. For example, in guinea pigs, the satellite sequence is CCCTAA.* Satellites are usually present at the centromeres of chromosomes. Their role is not known.

► Minisatellites are 12-100 base pairs long and are repeated several thousand times. Because DNA polymerase tends to slip and make errors in copying these sequences, they are variable in the numbers of copies. For

*When a DNA sequence such as CCCTAA is written, the complementary bases on the other strand are assumed.

example, one person might have 300, and another, 500. This variation provides a set of molecular genetic markers for identifying an individual. ► Microsatellites are very short (1-5 base pairs) sequences, present in small clusters of 10-50 copies. They are scattered all over the genome, and have been used in human gene sequencing.

While laboratory scientists have made use of these sequences in genetic studies, their roles in eukaryotes are not clear.

Telomeres are repetitive sequences at the ends of chromosomes

There are several types of moderately repetitive sequences in the eukaryotic genome. One type is important in maintaining the ends of chromosomes when DNA is replicated. Recall from Chapter 11 that replication proceeds differently on the two strands of a DNA molecule. Both new strands form in the 5'-to-3' direction, but one strand (the leading strand) grows continuously from one end to the other, while the other (the lagging strand) grows as a series of short Oka-zaki fragments (see Figure 11.17).

In a eukaryotic chromosome, replication must begin with an RNA primer at the 5' end of the forming strand. The leading strand can grow without interruption to the very end, but on the lagging strand there is nothing beyond the primer in the 5' direction to replace the RNA. So the new chromosome formed after DNA replication lacks a bit of double-stranded DNA at each end. This situation signals DNA repair mechanisms in the cell, and the single-stranded regions, along with some of the intact double-stranded end, is cut off. In this way, the chromosome becomes shorter with each cell division.

In many eukaryotes, there are moderately repetitive sequences at the ends of chromosomes called telomeres. In humans, the sequence is TTAGGG, and it is repeated about 2,500 times (Figure 14.3a). These repeats bind special proteins that maintain the stability of chromosome ends. Otherwise, the DNA rapidly breaks down.

(«)

© ©

© ©

(b)

© ©

© ©



GGGATTGGGATTGGGATTGGGATTGGGATTI₃

CCCTAACCTAACCTAACCTAACCTAArs

GGGATTGGGATTGGGATTGGGATTGGGATTI₃

CCCTAACCTAACCTAACCTAA

I©

GGGATTGGGATTGGGATTGGGATTGGGATTI₃

CCCTAACCTAACCTAACCTAA fs

RNA Telomerase

GGGATTGGGATTGGGATTGGGATTGGGATTI_{li}

CCCTAACCTAACCTAACCTAACCTAAD

Human telomeres have about 2,500 repeats of this sequence.

Because there is no primer at the extreme 5' end of a chromosome, there is a gap in replication, leading to shortening of the chromosome after each round of replication. Chromosome shortening leads in turn to cell death.



An RNA in telomerase acts as a template for DNA. This enzyme adds the telomeric sequence to the end of the chromosome.

The original length of the chromosomal DNA has been restored. Note the gap where the primer for DNA replication has been removed.

14.3 Telomeres and Telomerase

(a) The loss of moderately repetitive sequences from the telomere leads to cell death, (b) In cells that divide continuously (such as germ line cells), the enzyme telomerase prevents the loss of telomeric ends.

264 CHAPTER FOURTEEN

DNA

DNA

RNA

primary

transcript

When human cells are removed from the body and put in a nutritious medium in the laboratory, they will grow and divide. But each chromosome can lose 50-200 bp of telomeric DNA after each round of DNA replication and cell division. This shortening compromises the stability of the chromosomes. After 20-30 divisions, chromosomes are unable to take part in cell division, and the cell dies. The same thing happens in the body, and explains in part why cells do not last the entire lifetime of the organism: Their telomeres shorten.

Yet constantly dividing cells, such as bone marrow cells and germ line cells, manage to maintain their moderately repetitive telomeric DNA. An enzyme, appropriately called telomerase, prevents the loss of this DNA by catalyzing the addition of any lost telomeric sequences (Figure 14.3b). Telomerase is made up not only of proteins, but also of an RNA sequence that acts as a template for the telomeric sequence addition.

Considerable interest has been generated by the finding that telomerase is expressed in more than 90 percent of human cancers. Telomerase may be an important factor in the ability of cancer cells to divide continuously. Since most normal cells do not have this ability, telomerase is an attractive target for drugs designed to attack tumors specifically.

There is also interest in telomerase and aging. When a gene expressing high levels of telomerase is added to human cells in culture, their telomeres do not shorten, and instead of dying after 20-30 cell generations, the cells become immortal. It remains to be seen how this finding relates to the aging of a large organism.

Some moderately repetitive sequences are transcribed

Some moderately repetitive DNA sequences code for tRNA's and rRNA's, which are used in protein synthesis (see Chapter 12). These RNA's are constantly being made, but even at the maximum rate of transcription, single copies of these sequences would be inadequate to supply the large amounts of these molecules needed by most cells; hence there are multiple copies of the DNA sequences coding for them. Since these moderately repetitive sequences are transcribed into RNA, they are properly termed "genes," and we can speak of rRNA genes and tRNA genes. In mammals, there are four different rRNA molecules that make up the ribosome—the 18S, 5.8S, 28S, and 5S rRNA's.* The 18S, 5.8S, and 28S rRNA's are transcribed from a repeated sequence of DNA as a single precursor, which is twice the size of the three ultimate products (Figure 14.4). Several posttranscriptional steps cut this precursor into its final three rRNA's and discard the nonuseful, or "spacer," RNA. The DNA coding for these RNA's is moder-

*The measure "S" refers to the movement of a molecule in a centrifuge: In general, larger molecules have a higher S value.

The rRNA coding unit is repeated many times (280 in humans).

W H I I I U I I I

I I I I I 19 I I I I I

I I ■ H I I « H I I ■ ■

1:1 I I I I I ■ I I I

I I I I I H I H I ■ ■ ■

13,000 bp Transcribed region

30,000 bp

Nontranscribed

spacer region

18S 5.8S

28S

Processing steps splice out the spacers...]

18S 5.8S

28S

f ...resulting in three rRNAs.J

74.4 A Moderately Repetitive Sequence Codes for rRNA

This rRNA gene, along with its nontranscribed spacer, is repeated 280 times in the human genome.

ately repetitive in humans: A total of 280 copies of the sequence are located in clusters on five different chromosomes.

Other moderately repetitive sequences in mammals are not clustered, but instead are scattered throughout the genome. These DNA's usually are not transcribed and usually are short, about 300 bp long. In humans, half of these DNA's are of a single type, called the Alu family (because they contain a sequence that is recognized by a nuclease enzyme, Alu I). There are 300,000 copies of the Alu family in the genome, and they may act as multiple origins for DNA replication.

Transposable elements move about the genome

Most of the remaining scattered moderately repetitive DNA is not stably integrated into the genome. Instead, these DNA sequences can move from place to place in the genome. Such sequences are called transposable elements, or transposons.

There are four main types of transposable elements in eukaryotes:

► SINEs (short interspersed elements) are up to 500 bp long and are transcribed, but not translated.

► LINEs (long interspersed elements) are up to 7,000 bp long, and some are transcribed and translated into proteins. They constitute about 15 percent of the human genome.

Both of these elements are present in more than 100,000 copies. They move about the genome in a distinctive way: They make an RNA copy, which acts as a template for the

I The transposon carries a gene for transposase, which catalyzes the movements of the DNA.

^

Transposon

DNA

tim

EST

UjTransposase gene hrH

Inverted repeat Inverted repeat Protein-coding gene

DNA

Q Transposase allows the DNA to loop out and move to a new location in the genome.



§JThe transposon has inserted within a protein-coding gene, disrupting it...

Disrupted gene

Disrupted gene

DNA

...and resulting in a nonfunctional mRNA and/or protein.

74.5 Transposons and Transposition

At the end of each transposable element is an inverted repeat sequence that helps in the transposition process.

new DNA, which then inserts itself at a new location in the

► Retrotransposons also make an RNA copy when they move. They are rare in mammals, but are more common in other animals and yeasts. The genetic organization of viral retrotransposons resembles that of retroviruses such as HIV, but these segments lack the genes for protein coats and thus cannot produce viruses.

► DNA transposons are similar to their prokaryotic counterparts. They do not use an RNA intermediate, but actually move to a new spot in the genome without replicating (Figure 14.5).

What role do these moving sequences play in the cell? There are few answers to this question. The best answer so far seems to be that transposons are cellular parasites that simply replicate themselves. But these replications can lead to the insertion of a transposon at a new location, and this event has important consequences. For example, insertion of a transposon into the coding region of a gene causes a mutation because of the addition of new base pairs. This has been found in rare forms of several human genetic diseases, including hemophilia and muscular dystrophy.

If the insertion of a transposon takes place in the germ line, a gamete with a new mutation results. If the insertion takes place in a somatic cell, cancer may result. If a transposon replicates not just itself but also an adjacent gene, the result may be a gene duplication. A transposon can carry a

THE EUKARYOTIC GENOME AND ITS EXPRESSION 265

gene, or a part of it, to another location on a chromosome, shuffling genetic material and creating new genes. Clearly, transposition stirs the genetic pot in the eukaryotic genome and thus contributes to genetic variability.

In Chapter 4, we described the endosymbiosis theory of the origin of chloroplasts and mitochondria, which proposes that these organelles are the descendants of once free-living prokaryotes. Transposable elements may have played a role in this process. In living eukaryotes, although the organelles have some DNA, the nucleus contains most of the genes that encode the organelle proteins. If the organelles were once independent, they must originally have contained all of these genes. How did the genes move to the nucleus? The answer may lie in DNA transposition. Genes once in the organelle may have moved to the nucleus by well-known molecular events that still occur today. The DNA that remains in the organelles may be the remnants of more complete prokaryotic genomes.

The Structures of Protein-Coding Genes

Like their prokaryotic counterparts, many protein-coding genes in eukaryotes are single-copy DNA sequences. But eukaryotic genes have two distinctive characteristics that are uncommon among prokaryotes. First, they contain non-coding internal sequences, and second, they form gene families with structurally and functionally related cousins in the genome.

Protein-coding genes contain noncoding internal and flanking sequences

Preceding the coding region of a eukaryotic gene is a promoter, where RNA polymerase begins the transcription process. Unlike the prokaryotic enzyme, eukaryotic RNA polymerase does not recognize the promoter sequence by itself, but requires help from other molecules, as we'll see later. At the other end of the gene, after the coding region, is a DNA sequence appropriately called the terminator, which RNA polymerase recognizes as the end point for transcription (Figure 14.6). Neither the promoter nor the terminator sequence is transcribed into RNA.

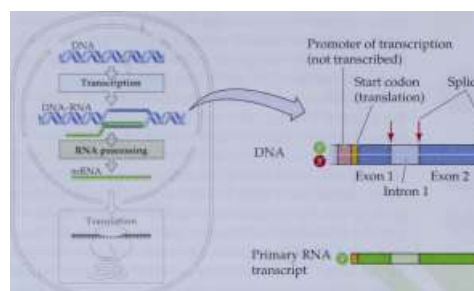
Eukaryotic protein-coding genes also contain noncoding base sequences, called introns. One or more introns are interspersed with the coding regions—called exons—in most eukaryotic genes. Transcripts of the introns appear in the primary transcript of RNA—the pre-mRNA—within the nucleus, but by the time the mature mRNA exits the organelle, they have been removed. The transcripts of the introns are cut out of the pre-mRNA, and the transcripts of the exons are spliced together.

The locations of the introns can be determined by comparing the base sequences of a gene (DNA) with those of its final mRNA. Although direct sequencing of the DNA that codes for an mRNA is the easiest way to map the locations of introns within a gene, nucleic acid hybridization is the method that originally revealed the existence of introns in protein-coding genes. This method, outlined in

266 CHAPTER FOURTEEN

Promoter of transcription (not transcribed)

Splice sites



Terminator of transcription (not transcribed)

Stop codon (translation)

J Li

G

©

Intron 2

Exon 3

Q The exons and introns of the coding region are both transcribed.

74.6 The Structure and Transcription of a Eukaryotic Gene

The P-globin gene is about 1,600 bp long. The exons—DNA-coding sequences— contain 441 base pairs (triplet codons for 146 amino acids plus a triplet stop codon). Noncoding sequences of DNA—introns—are initially transcribed between codons 30 and 31 (130 bp long) and 104 and 105 (850 bp long), but are spliced out of the final transcript.

Figure 14.7, has been crucial to genetic research; in later chapters we will see its use in localizing genes, testing for alleles, localizing mRNA's during development, and many other applications.

mRNA r_r



I©

The introns are removed.

The spliced exons are ready for translation.

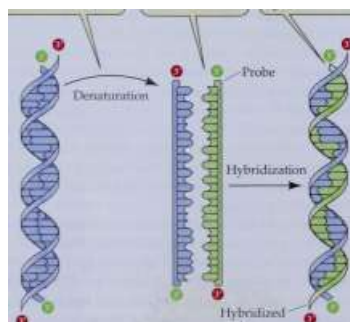
RESEARCH METHOD

Upon being carefully heated, the two strands of a DNA molecule denature (separate).

If a probe made of single-stranded DNA or RNA is added to the denatured DNA...

...it binds the template DNA strand, forming double-stranded

hybrid molecules.



probe

To examine the relationship between a gene and its transcript, biologists used nucleic hybridization to examine the gene for one of the globin proteins that make up hemoglobin (Figure 14.8). They first denatured the globin DNA by heating it, then added mature globin mRNA. As expected, the mRNA bound to the DNA by complementary base pairing. The researchers expected to obtain a linear matchup of the mRNA to the globin-coding DNA. They got their wish, in part: There were indeed stretches of RNA-DNA hybridization. But some looped structures were also visible. These loops were the introns, stretches of DNA that did not have complementary bases on the mRNA. Later studies showed that hybridization to the gene using pre-mRNA was complete, and that the introns were indeed transcribed. Somewhere on the path from transcript to mature mRNA, the introns had been removed, and the exons had been spliced together. We will examine this splicing process later in the chapter.

Most (but not all) vertebrate genes contain introns, as do many other eukaryotic genes (and even a few prokaryotic ones). Introns interrupt, but do not scramble, the DNA sequence that codes for a polypeptide chain. The base sequence of the exons, taken in order, is exactly complementary to that of the mature mRNA product. The introns, therefore, separate a gene's protein-coding region into distinct parts—the exons. In some cases, the separated exons code for different functional regions, or domains, of the protein. For example, the globin proteins that make up hemoglobin have two domains: one for binding to heme, and another for binding to the other globin chains. These two domains are coded for by different exons in the globin gene.

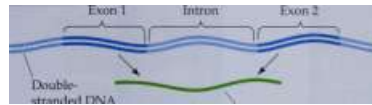
74.7 Nucleic Acid Hybridization

Base pairing permits the detection of a sequence complementary to the probe.

EXPERIMENT

Question: Are there regions within the coding sequence of a gene that do not end up in its mRNA?

METHOD



Double-stranded DNA

| Mouse DNA is partially denatured and hybridized to mRNA from a mouse gene.

RESULTS

Introns present

Globin mRNA transcribed from exons 1 and 2



y

■ -v;



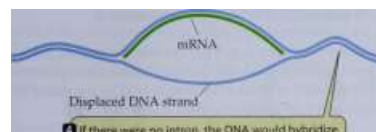
Q Interpretation of electron microscopy: The mRNA has hybridized with the template DNA of its gene, forming thick double strands. The thin loops were the nontemplate (other) strand of DNA that was displaced by the mRNA.



\

The double-stranded intron is forced into a loop by the mRNA, bringing the two exons together.

If there were no intron:



I If there were no intron, the DNA would hybridize with the mRNA in a continuous strand with no double-stranded loop present.

Conclusion: The DNA contains noncoding internal regions within the genes that are not present in the final mRNA.

Many eukaryotic genes are members of gene families

About half of all eukaryotic protein-coding genes are present in only one copy in the haploid genome. The rest have multiple copies. Often, inexact, nonfunctional copies of a particular gene, called pseudogenes, are located near it on a chromosome. These duplicates may have arisen by an ab-

THE EUKARYOTIC GENOME AND ITS EXPRESSION 267

74.8 Hybridization Revealed Noncoding DNA

When an mRNA transcript was experimentally hybridized to the double-stranded DNA of a gene, the introns from the DNA "looped out," demonstrating that the coding region of a eukaryotic gene can contain noncoding DNA that is not present in the mRNA transcript.

normal event in chromosomal crossing over during meiosis or by the action of retrotransposons.

In other cases, however, the genome contains slightly altered copies of a gene that are functional. A set of duplicated or related genes is called a gene family. Some families, such as the β -globins that are part of hemoglobin, contain only a few members; other families, such as the immunoglobulins that make up antibodies, have hundreds of members.

Like the members of any family, the DNA sequences in a gene family are usually different from one another to a certain extent. As long as one member retains the original DNA sequence and thus codes for the proper protein, the other members can mutate slightly, extensively, or not at all. The availability of such extra genes is important for "experiments" in evolution: If the mutated gene is useful, it may be selected for in succeeding generations. If the gene is a total loss (a pseudogene), the functional copy is still there to save the day.

The gene family for the globins is a good example of the gene families found in vertebrates. These proteins are found in hemoglobin, as well as in myoglobin (an oxygen-binding protein present in muscle). The globin genes probably all arose from a single common ancestor gene long ago. In humans, there are three functional members of the α -globin (α -globin) cluster and five in the β -globin (β -globin) cluster (Figure 14.9). In a human adult, each hemoglobin molecule is a tetramer containing the heme pigments (each held inside a globin polypeptide), two identical α -globins, and two identical β -globins.

Spacer DNA is noncoding DNA between gene family members.

[β -Globin gene cluster

Gy

Ay

vPi

α -Globin gene cluster

Pseudogenes are family members that do not code for functional mRNAs or proteins.



\\iC,l v|/al

al

74.9 Gene Families

The human α -globin and β -globin gene clusters are located on different chromosomes. Each family is organized into a cluster of genes separated by noncoding "spacer" DNA. The nonfunctional pseudogenes are indicated by the Greek letter psi (ψ).

268 CHAPTER FOURTEEN

14.10 Differential Expression in the β -Globin Gene Family

During human development, different members of the β -globin gene family are expressed at different times and in different tissues.

During human development, different members of the β -globin gene family are expressed at different times and in different tissues (Figure 14.10). This differential gene expression has great physiological significance. For example, γ -globin, a subunit found in the hemoglobin of the fetus ($\alpha^2\gamma_2$), binds O_2 more tightly than adult hemoglobin ($\alpha_2\beta_2$) does. (Both γ -globin and β -globin are members of the β -globin family.) This specialized form of hemoglobin ensures that in the placenta, where the maternal and fetal circulation come near each other, O_2 will be transferred from the mother's to the developing child's blood. Just before birth, the synthesis of fetal hemoglobin in the liver stops, and the bone marrow cells take over, making the adult form.

24-week-old fetus

18-week-old baby

50 -

c

40

I 30

IS

o

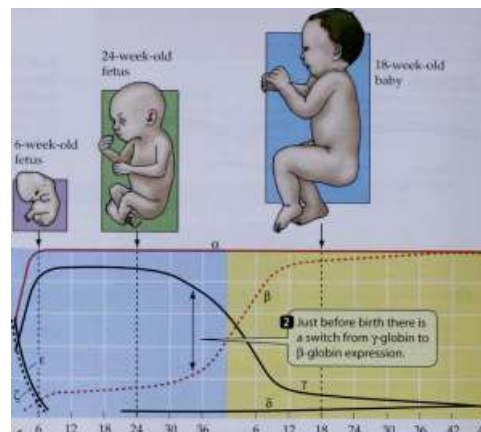
"5b 20

"re

I 10

° 0

Q Different globin genes are expressed at different times during human development



ii

12 18 24

Number of weeks

after conception

Birth

18 24 30 36 Number of weeks after birth



Bone marrow

Fetal yolk sac

Fetal spleen

f

The vertical dimension of these shapes represents the relative expression of the globin genes in different tissues.

In addition to genes that encode proteins, the globin family includes nonfunctional pseudogenes, designated with the Greek letter psi ((p). These pseudogenes are the "black sheep" of any gene family: They result from mutations that cause a loss of function rather than an enhanced or new function.

The DNA sequence of a pseudogene may not differ vastly from that of other family members. It may just lack a promoter, for example, and thus cannot be transcribed. Or it may lack the recognition sites for the removal of introns, and thus will be transcribed into pre-mRNA, but not correctly processed into a useful mRNA. In some gene families, pseudogenes outnumber functional genes. However,

since some members of the family are functional, there appears to be little selective pressure in evolution to eliminate pseudogenes.

RNA Processing

As we have seen, the primary RNA transcript (pre-mRNA) of a eukaryotic gene is not the same as the mature mRNA. To produce the mRNA, the primary transcript is processed by the addition of bases at both ends, and by the removal of introns and the joining of exons.



DNA-RNA ^ « _



t

A "cap" of modified GTP is added here.

©

RNA primary transcript

o

G cap —CD

Coding region of

primary transcript a

Processed primary RNA transcript

tThis sc byRN,

This sequence is recognized by RNA cleavage enzyme.

=/f=

77



T

RNA is cut here, and a poly A "tail" is added.

8| This symbol indicates that a large piece of RNA is not shown. It may be thousands of bases long.

14.11 Processing the Ends of Eukaryotic mRNA

The modifications at both ends—the "cap" and the "tail"—are important for mRNA function.

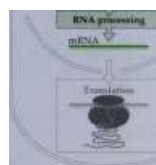
DNA

Transcription

DNA-RN \

JOwtt m. ■./ OfIod

III •' _~l.



Primary mRNA transcript

^ snRNP 5' Exon ^ <±=i.>



IjThe small ribonucleo-protein (snRNP) particle binds at the 5' splice site and a second binds near the 3' splice site.

©I

Intron

r

2EC

snRNP

3' Exon

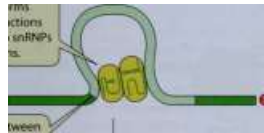
5' Splice site

Q A spliceosome forms because of interactions between the two snRNPs and other proteins.

3' Splice site

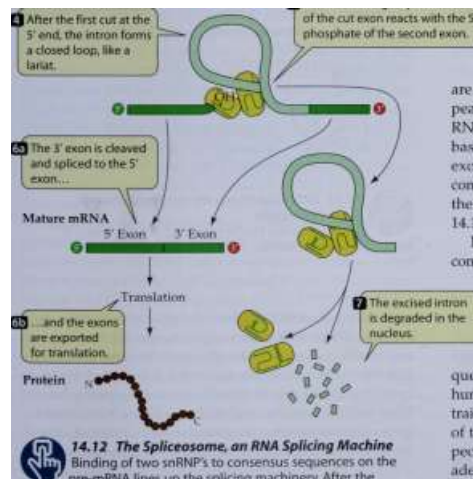
©

Q A cut is made between the 5' exon and the intron.



Q After the first cut at the 5' end, the intron forms a closed loop, like a lariat.

Q The free 3' OH group at the end of the cut exon reacts with the 5' phosphate of the second exon.



14.12 The Spliceosome, an RNA Splicing Machine

Binding of two snRNP's to consensus sequences on the pre-mRNA lines up the splicing machinery. After the snRNP's bind to pre-mRNA, other proteins join the complex to form a spliceosome.

THE EUKARYOTIC GENOME AND ITS EXPRESSION 269

The primary transcript of a protein-coding gene is modified at both ends

Two early steps in the processing of pre-mRNA are the addition of a "cap" at the 5' end and the addition of a "tail" at the 3' end (Figure 14.11).

The G cap is a chemically modified molecule of guano-sine triphosphate (GTP). It is added to the 5' end as the RNA is transcribed. The cap apparently facilitates the binding of mRNA to the ribosome for translation and protects the mRNA from breaking down.

The poly A tail is added to the 3' end of pre-mRNA after a terminal sequence has been removed. Near the 3' end of pre-mRNA, and after the last codon, is the sequence AAUAAA. This sequence acts as a signal for an enzyme to cut the pre-mRNA. Immediately after this cleavage, another enzyme adds 100 to 300 residues of adenine (poly A) to the 3' end of the pre-mRNA. This tail may assist in the export of the mRNA from the nucleus.

Splicing removes introns from the primary transcript

The next step in processing of eukaryotic pre-mRNA within the nucleus is deleting the introns. If these RNA regions were not removed, a nonfunctional mRNA, producing an improper amino acid sequence and thus a nonfunctional protein, would result. The process called RNA splicing removes the introns and splices the exons together.

As soon as the pre-mRNA is transcribed, it is quickly bound to several small nuclear ribonucleo-protein particles (snRNP's, commonly pronounced "snurps"). There are several types of these RNA-protein particles in the nucleus.

At the boundaries between introns and exons are consensus sequences —short stretches of DNA that appear, with little variation, in many different genes. The RNA in one of the snRNP's (called U1) has a stretch of bases complementary to the consensus sequence at the 5' exon-intron boundary, and binds to the pre-mRNA by complementary base pairing. Another snRNP (U2) binds to the pre-mRNA near the 3' intron-exon boundary (Figure 14.12).

Next, other proteins bind, forming a large RNA-protein complex called a spliceosome. The spliceosome uses energy from ATP for its assembly. It cuts the RNA, releases the introns, and joins the ends of the exons together to produce mature mRNA.

Molecular studies of human diseases have been valuable tools in the investigation of consensus sequences and splicing machinery. Beta thalassemia is a human genetic disease inherited as an autosomal recessive trait. People with this disease make an inadequate amount of the β -globin subunit that is part of hemoglobin. These people suffer from severe anemia because they have an inadequate supply of red blood cells. In some cases, the genetic mutation that causes the disease occurs at a consensus sequence in the β -globin gene. Consequently, the pre-

270 CHAPTER FOURTEEN

mRNA cannot be-spliced correctly, and nonfunctional β -globin mRNA is made.

This finding is an excellent example of the use of mutations in determining a cause-and-effect relationship in biology. In the logic of science, merely linking two phenomena (for example, consensus sequences and splicing) does not prove that one is necessary for the other. In an experiment, the scientist alters one phenomenon (for example, the base sequence at the consensus region) to see whether the other event (for example, splicing) occurs. In beta thalassemia, nature has done the experiment for us.

After the processing events are completed in the nucleus, the mRNA exits the organelle, apparently through the nuclear pores (see Figure 4.9). A receptor at the nuclear pore recognizes the processed mRNA (or a protein bound to it). Unprocessed or incompletely processed pre-mRNA's remain in the nucleus.

Transcriptional Control

In a multicellular organism with specialized cells and tissues, every cell contains every gene in the organism's genome. For development to proceed normally, and for each cell to acquire and maintain its proper function, certain proteins must be synthesized at just the right times and in just the right cells. Thus, the expression of eukaryotic genes must be precisely regulated.

Regulation of gene expression can occur at many points (Figure 14.13). This section describes the mechanisms that control the transcription of specific genes. These often involve nuclear proteins that alter chromosome function or structure. In some cases, the regulation of transcription involves changes in the DNA itself: Genes are selectively replicated to give more templates to transcribe, or even rearranged on the chromosome.

Posttranscriptional events can also regulate gene expression. As we have seen, the processing of pre-mRNA can be controlled after transcription. The transport of the mRNA into the cytoplasm, and how long it remains there, can also be controlled. The translation of mRNA into protein can also be regulated. Finally, once the protein itself is made, its structure can be modified, or it can be broken down and destroyed.

Specific genes can be selectively transcribed

The brain cells and the liver cells of a mouse have some proteins in common and some that are distinctive for each cell type. Yet both cells have the same DNA sequences and, therefore, the same genes. Are the differences in protein content due to differential transcription of genes? Or is it that all the genes are transcribed in both cell types, and a posttranscriptional mechanism is responsible for the differences in proteins?

These two alternatives—transcriptional or posttranscriptional control—can be distinguished by examination of the actual RNA sequences made within the nucleus of each cell type. Such analyses indicate that for some proteins, the

DNA

f⁺/W⁺f⁺/WWWi

Transcriptional control

SiM/H

HI II I I H III II II I I



H/W

\

Primary RNA transcript

Nucleus

Processing control

mRNA

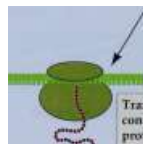
mtmmmmmmmm

Cytoplasm

Transport control

mRNA

mtmmmmmmimm



mRNA

stability

control

Translational control of protein synthesis

mtmmmmmmmm

Inactive mRNA

Posttranslational control of protein function



I pNsS 1 ^ Active/inactive \ ^>***o protein

fn^ 14.13 Potential Points for the Regulation of Gene Expression

Gene expression can be regulated at three levels: at transcription, at translation, or after translation.

mechanism of control is differential gene transcription. Both brain and liver cells, for example, transcribe "housekeeping" genes, such as those for glycolysis enzymes and ribosomal RNA's. But liver cells transcribe some genes for liver-specific proteins, and brain cells transcribe some genes for brain-specific proteins. And neither cell type transcribes the genes for proteins that are characteristic of muscle, blood, bone, and the other specialized cell types in the body.

CONTRASTING EUKARYOTES AND PROKARYOTES. Unlike pro-

karyotes, in which related genes are transcribed as a unit in

operons, eukaryotes tend to have solitary genes. Thus, regulating several genes at once requires common control elements in each gene, which allow all of the genes to respond to the same signal.

In contrast to the single RNA polymerase in bacteria, eukaryotes have three different RNA polymerases. Each eukaryotic polymerase catalyzes the transcription of a specific type of gene. Only one (RNA polymerase II) transcribes protein-coding genes to mRNA. The other two transcribe the DNA that codes for rRNA (polymerase I) and for tRNA and small nuclear RNA's (polymerase III). The diversity of eukaryotic polymerases is reflected in the diversity of eukaryotic promoters, which tend to be much more variable than prokaryotic promoters. In addition, most eukaryotic genes have regulator, enhancer, and silencer elements (which we will discuss shortly) that can control the rate of transcription. Whether a eukaryotic gene is transcribed depends on the sum total of the effects of all of these DNA and protein elements; thus there are many points of possible control.

Finally, the transcription complex in eukaryotes is very different from that of prokaryotes, in which a single peptide subunit

can cause RNA polymerase to recognize the promoter. In eukaryotes, many proteins are involved in initiating transcription. We will confine the following discussion to RNA polymerase II, which catalyzes the transcription of most protein-coding genes, but the mechanisms for the other two polymerases are similar.

transcription FACTORS. In prokaryotes, the promoter is a sequence of DNA near the 5' end of the coding region of a gene where RNA polymerase begins transcription. A prokaryotic promoter has two essential regions. One is the recognition sequence —the sequence recognized by RNA polymerase. The second, closer to the initiation point, is the TATA box (so called because it is rich in AT base pairs), where DNA begins to denature so that its templates can be exposed. In eukaryotes, there is a TATA box about 25 bp away from the initiation site for transcription, and one or two recognition sequences of about 50 to 70 bp 5' from the TATA box.

Eukaryotic RNA polymerase II cannot simply bind tightly to the promoter and initiate transcription. Rather, it binds and acts only after various regulatory proteins, called transcription factors, have assembled on the chromosome (Figure 14.14). First, the protein TFIID ("TF" stands for transcription /actor) binds to the TATA box. Its binding changes both its own shape and that of the DNA, presenting a new surface that attracts the binding of other transcription factors. RNA polymerase II does not bind until several other proteins have already bound to this complex.

Some DNA sequences, such as the TATA box, are common to the promoters of many genes and are recognized by transcription factors that are found in all the cells of an organism. Other sequences in promoters are specific to only a few genes and are recognized by transcription factors found only in certain tissues. These specific transcription

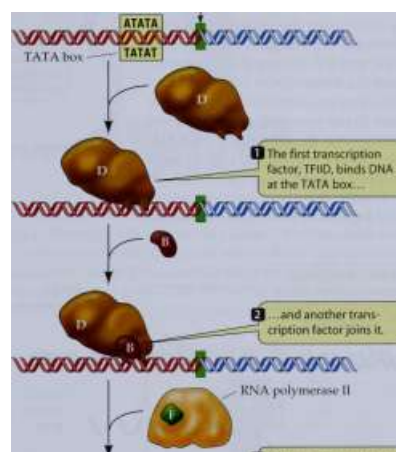
factors play an important role in differentiation, the specialization of cells during development.

REGULATORS, ENHANCERS, AND SILENCERS IN DNA. In addition to the promoter, two other regions of DNA bind proteins that activate RNA polymerase. The recently discovered regulator regions are clustered just upstream of the promoter. Various regulator proteins (seven in the [3-globin

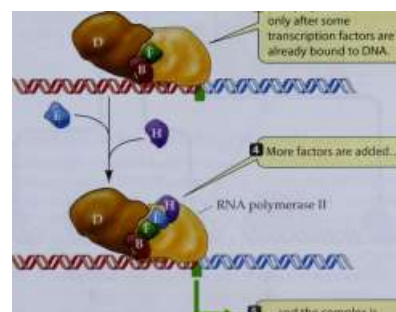
Promoter

a

Starting point for transcription



Q RNA polymerase binds } only after some transcription factors are already bound to DNA.



-c

..and the complex is ready to transcribe RNA.



4 74.74 The Initiation of Transcription in Eukaryotes

Except for TFIID, which also binds to the TATA box, each transcription factor has binding sites only for the other proteins and does not bind directly to DNA.

74.75 The Roles of Transcription Factors, Regulators, and Activators

The actions of many proteins determine whether and where RNA polymerase II will transcribe DNA.

gene) may bind to these regions (Figure 14.15). Their net effect is to bind to the adjacent transcription complex and activate it.

Much farther away—up to 20,000 bp away—are the enhancer regions. Enhancer regions bind activator proteins, and this binding strongly stimulates the transcription complex. How enhancers can exert this influence is not clear. In one proposed model, the DNA bends—it is known to do so—so that the activator is in contact with the transcription complex (see Figure 14.15).

Finally, there are negative regulatory regions on DNA called silencers,

Q A stressor (e.g., heat) causes the activation of transcription of a gene for stress proteins.

Enhancer

1j I

-Activator protein

Regulator protein

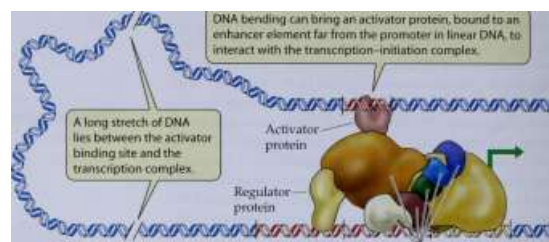
Regulator Transcription RNA Transcribed protein factor polymerase region binding binding binding ►-

z RNA

polymerase II

\

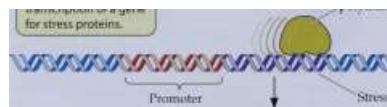
DNA bending can bring an activator protein, bound to an enhancer element far from the promoter in linear DNA, to interact with the transcription-initiation complex.



»

RNA

polymerase



Promoter

o Binding of stress proteins to the stress response element sequence stimulates transcription of genes A, B, and C..

ikaWW T

Q

Q

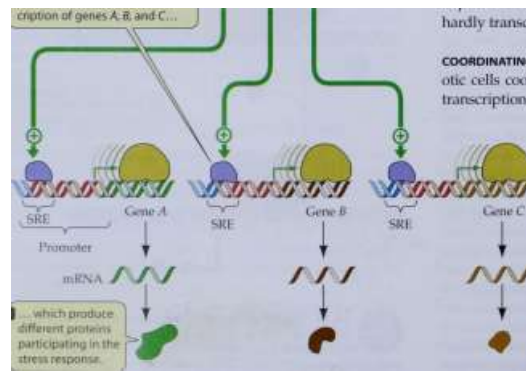
a

Stress

protein

gene

- Stress proteins



§|... which produce different proteins participating in the stress response.

Transcription factors

which have the reverse effect of enhancers. Silencers turn off transcription by binding proteins appropriately called repressors.

How do these proteins and DNA sequences—transcription factors, activators, repressors, regulators, enhancers, and silencers—regulate transcription? Apparently, all genes in most tissues can transcribe a small amount of RNA. But the right combination of the factors is what determines the maximum rate of transcription. In the immature red blood cells of bone marrow, for example, which make a large amount of α -globin, the transcription of globin genes is stimulated by the binding of 7 regulators and 6 activators. But in white blood cells in the same bone marrow, these 13 proteins are not made, and they do not bind to their sites adjacent to the (α -globin genes; consequently, these genes are hardly transcribed at all.

COORDINATING THE EXPRESSION OF GENES. How do eukary-

otic cells coordinate the regulation of several genes whose transcription must be turned on at the same time? In prokaryotes, in which related genes are linked together in an operon, a single regulatory system can regulate several adjacent genes. But in eukaryotes, the several genes whose regulation requires coordination may be on different chromosomes.

14.16 Coordinating Gene Expression

A single signal, for example heat stress, causes the synthesis of a transcriptional regulator for many genes.

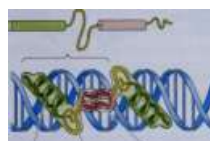
THE EUKARYOTIC GENOME AND ITS EXPRESSION 273

In such a case, regulation can be achieved if the various genes all have the same regulatory sequences near them, which bind to the same activators and regulators. One of the many examples of this phenomenon is provided by the response of organisms to a stressor—for example, drought in plants. Under conditions of drought stress, a plant must synthesize various proteins, but the genes for these proteins are scattered throughout the genome. However, each of these genes has a specific regulatory sequence near its promoter called the stress response element (SRE). The binding of a regulator protein to this element stimulates RNA synthesis (Figure 14.16). In the drought example, the proteins made are involved not only in water conservation, but also in protecting the plant against excess salt in the soil and against freezing. This finding has considerable importance for agriculture, in which crops are often grown under less than optimal conditions.

the binding of proteins to dna. A key to transcriptional control in eukaryotes is that transcription factors, regulators, activators, and repressors all bind to specific DNA sequences. In these proteins, there are four common structural themes in the domains that bind to DNA. These themes are called motifs and consist of combinations of structures and special components.

The helix-turn-helix motif involves several α -helices, one of which makes contact with DNA; the others stabilize the structure. This motif appears in the proteins that activate genes involved in embryonic development (homeobox proteins; see Chapter 16) and in the proteins that regulate the development of the immune and central nervous systems.

Helix-turn-helix motif

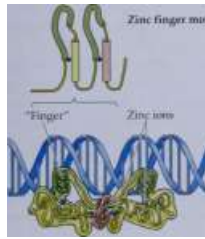


DNA-binding helix Turn

Dimer-binding helix

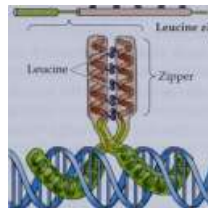
The zinc finger motif has loops that form when a zinc ion is held by the amino acids cysteine and histidine. It occurs most notably in the receptors for steroid hormones (see Figure 15.9).

Zinc finger motif



The leucine zipper motif places hydrophobic leucine residues on one side of a polypeptide. Their presence allows two polypeptide chains to interact (zipper) hydrophobically, setting up the positively charged residues just past the zipper to bind to DNA. This motif occurs in many DNA-binding proteins—for example, the transcription factor AP-1, which binds near promoters of genes involved in mammalian cell growth and division. Overactivity of AP-1 has been linked to several types of cancer.

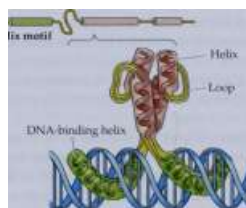
> Leucine zipper motif



The helix-loop-helix motif is two helices separated by a loop. This region is adjacent to a stretch of amino acids that interact with DNA. This motif occurs in the activator proteins that bind to enhancers for the immunoglobulin genes that synthesize antibodies, as well as in the transcription factors involved in muscle protein synthesis.

Helix-loop-helix motif

Helix



Genes can be inactivated by chromatin structure

Chromatin contains nucleosomes and many other chromosomal proteins (see Chapter 9). The packaging of DNA by these nuclear proteins can make DNA physically inaccessible to RNA polymerase and the rest of the transcription apparatus, much as the binding of a repressor to the operator in the prokaryotic lac operon prevents transcription. Both local and global chromatin structure affect transcription.

LOCAL EFFECTS. Nucleosomes inhibit both the initiation and elongation of transcription. To alleviate these blocks, cells recruit two protein complexes. One binds upstream of the initiation site, disaggregating the nucleosomes so that the large initiation complex can bind and begin transcription. The other binds once transcription is under way,

274 CHAPTER FOURTEEN

allowing the transcription complex to move through these nucleosomes. These processes are called chromatin remodeling (Figure 14.17).

global effects. Two kinds of chromatin can be distinguished by staining of the interphase nucleus: euchromatin and heterochromatin. Euchromatin is diffuse and stains lightly; it contains the DNA that is transcribed into mRNA. Heterochromatin stains densely and is generally not transcribed; any genes that it contains are thus inactivated. Perhaps the most dramatic example of heterochromatin is the inactive X chromosome of mammals.

A normal female mammal has two X chromosomes; a normal male, an X and a Y. The Y chromosome has only a few genes that are also present on the X, and is largely transcriptionally inactive in most cells. So there is a great difference between females and males in the "dosage" of X chromosome genes. In other words, each female cell has two copies of the genes on the X chromosome, and therefore has the potential to produce twice as much protein product of these genes as a male has. Yet X-linked gene expression is generally the same in males and females. How can this happen?

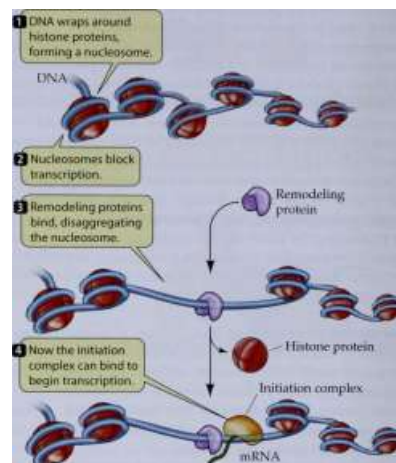
The answer was found in 1961 independently by Mary Lyon, Liane Russell, and Ernest Beutler. They suggested that one of the X chromosomes in each cell of an XX female is transcriptionally inactivated early in embryonic development. That copy of the X remains inactive in that cell, and in all the cells arising from it. In a given cell, the "choice" of which X in the pair of Xs to inactivate is usually random. Recall that one of the Xs in a female comes from her father and one from her mother. Thus, in one embryonic cell, the paternal X might be the one remaining active in mRNA synthesis, but in a neighboring cell, the maternal X might be active.

Interphase cells of XX females have a single, stainable nuclear body called a Barr body, after its discoverer, Murray Barr (Figure 14.18). This clump of heterochromatin, which is not present in males, is the inactivated X chromosome. The number of Barr bodies in each nucleus is equal to the number of X chromosomes minus one (the one represents the X chromosome that remains transcriptionally active). So a female with the normal two X chromosomes will have one Barr body, one with three X's will have two, an XXXX female will have three, and an XXY male will have one. We may infer that the interphase cells of each person, male or female, have a single active X chromosome, making the dosage of the expressed X chromosome genes constant across both sexes.

The mechanism of X inactivation involves chromosome condensation that makes the DNA sequences physically unavailable to the transcription machinery. One method may be the addition of a methyl group ($-\text{CH}_3$) to the 5' portion of cytosine on DNA. Such methylation seems to be

^»st prevalent in transcriptionally inactive genes. For ex-ai pie, most of the DNA of the inactive X chromosome has many cytosines methylated, while few of them on the ac-

INITIATION OF TRANSCRIPTION

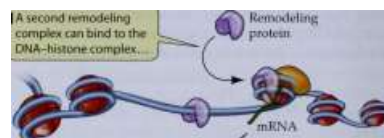


ELONGATION

IA second remodeling complex can bind to the DNA-histone complex..

mRNA

N Remodeling protein



f

...allowing transcription without disaggregation.

14.17 Local Remodeling of Chromatin for Transcription

Initiation of transcription requires that nucleosomes disaggregate. During elongation, however, they remain intact.

five X are methylated. Methylated DNA appears to bind certain chromosomal proteins, which may be responsible for heterochromatin formation. But this seems to occur after the actual inactivation event, making methylation a way to keep genes turned off.

The otherwise inactive X chromosome has one gene that is only lightly methylated and is transcriptionally active. This gene is called XIST (for X inactivation specific transcript), and it is heavily methylated on, and not transcribed from, the other, "active" X chromosome. The RNA transcribed from XIST does not leave the nucleus and is not an mRNA. Instead, it appears to bind to the X chromosome that transcribes it, and this binding somehow leads to a spreading of inactivation along the chromosome.

The Barr body is the condensed, inactive member of a pair of X chromosomes in the cell. The other X is not condensed and is active in transcription.

14.18 Barr Bodies in the Nuclei of Female Cells

The number of Barr bodies per nucleus is equal to the number of X chromosomes minus one. Thus males (XY) have no Barr body, whereas females (XX) have one.

A DNA sequence can move to a new location to activate transcription

In some instances, gene expression is regulated by the movement of a gene to a new location on the chromosome. An example of this mechanism is found in the yeast *Saccharomyces cerevisiae*. The haploid single cells of this fungus exist in two mating types, *a* and *α*, which fuse to form a diploid zygote. Although all yeast cells have an allele for each of these types, the allele that is expressed determines the mating type of the cell. In some yeasts, the mating type changes with almost every cell division cycle. How does it change so rapidly?

The yeast cell keeps the two different alleles (coding for type *a* and type *α*) at separate locations on its chromosome, away from a third site, the MAT locus. The two mating type alleles are usually transcriptionally silent because a repressor protein binds to them. However, when a copy of the *a* or *α* allele is inserted at the MAT region, the gene for proteins of the appropriate mating type is transcribed.

A change in mating type requires three steps:

► First, a new DNA copy of the nonexpressed allele is made (if the cell is now *a*, the new copy will be the *α* allele).

THE EUKARYOTIC GENOME AND ITS EXPRESSION 275

► Second, the current occupant of the MAT region (in this case, the *a* DNA) is removed by an enzyme.

► Third, the new allele (*α*) is inserted at the MAT region and transcribed. The *a* proteins are now made, and the mating type is changed.

DNA rearrangement is also important in producing the highly variable proteins that make up the human repertoire of antibodies, and in cancer, when inactive genes move to be adjacent to active promoters.

Selective gene amplification results in more templates for transcription

Another way for one cell to make more of a certain gene product than another cell does is to have more copies of the appropriate gene and to transcribe them all. The process of creating more copies of a specific gene in order to increase transcription is called gene amplification.

As described earlier, the genes that code for three of the four human ribosomal RNA's are linked together in a unit, and this unit is repeated several hundred times in the genome to provide multiple templates for rRNA synthesis (rRNA is the most abundant kind of RNA in the cell). In some circumstances, however, even this moderate repetition is not enough to satisfy the demands of the cell. For example, the mature eggs of frogs and fishes have up to a trillion ribosomes. These ribosomes are used for the massive protein synthesis that follows fertilization. The cell that differentiates into the egg contains fewer than 1,000 copies of the rRNA gene cluster, and would take 50 years to make a trillion ribosomes if it transcribed those rRNA genes at peak efficiency. How does the egg end up with so many ribosomes (and so much rRNA)?

The egg cell solves this problem by selectively amplifying its rRNA gene clusters until there are more than a million copies. In fact, this gene complex goes from being 0.2 percent of the total genome DNA to being 68 percent. These million copies transcribed at maximum rate (Figure 14.19) are just enough to make the necessary trillion ribosomes in a few days.

Strands of rRNA



Transcription begins here..



...the RNA elongates..

...and elongates until it is released here.

• - - *u

■ r •

DNA

\

i^ffj

* 'kv ..'



Multiple rRNA genes are actively transcribing rRNA precursors.

14.19 Transcription from Multiple Genes for rRNA

Elongating strands of rRNA transcripts form arrowhead-shaped regions, each centered on a strand of DNA that codes for rRNA.

276 CHAPTER FOURTEEN

The mechanism for selective overreplication of a single gene is not clearly understood, but it has important medical implications. As Chapter 18 will show, in some cancers, a cancer-causing gene called an oncogene becomes amplified. Also, in some tumors treated with a drug that targets a single protein, amplification of the gene for the target protein leads to an excess of that protein, and the cell becomes resistant to the prescribed dose of the drug.

Posttranscriptional Control

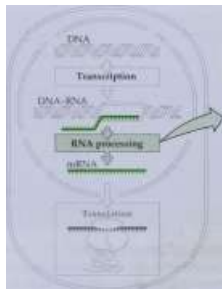
There are many ways to regulate the presence of mature mRNA in a cell even after a precursor has been transcribed. As we saw earlier, pre-mRNA can be processed by cutting out the introns and splicing the exons together. If the exons of the pre-mRNA are recombined in different ways by alternate splicing, different proteins can be synthesized. The longevity of mRNA in the cytoplasm can also be regulated. The longer an mRNA exists in the cytoplasm, the more of its coded protein can be made.

Different mRNA's can be made from the same gene by alternate splicing

Most primary transcripts contain several introns (see Figure 14.6). We have seen how the splicing mechanism recognizes the boundaries between exons and introns. What would happen if the (3-globin pre-mRNA, which has two introns, was spliced from the start of the first intron to the end of the second? Not only the two introns but also the middle exon would be spliced out. An entirely new protein (certainly not a p-globin) would be made, and the functions of normal (3-globin would be lost.

Alternate splicing can be a deliberate mechanism for generating a family of different proteins from a single gene. For example, a single pre-mRNA for the structural protein tropomyosin is alternatively spliced to give five different mRNA's and five different forms of tropomyosin found in five different tissues: skeletal muscle, smooth muscle, fibroblast, liver, and brain (Figure 14.20).

Primary RNA transcript for tropomyosin: 11 exons



The stability of mRNA can be regulated

DNA, as the genetic material, must remain stable, and there are elaborate mechanisms for repairing it if it becomes damaged. RNA has no such repair system. After it arrives in the cytoplasm, mRNA is subject to breakdown catalyzed by ribonucleases, which exist both in the cytoplasm and in lysosomes. But not all eukaryotic mRNA's have the same life span. Differences in the stabilities of mRNA's provide another mechanism for posttranscriptional control of protein synthesis. The less time an mRNA spends in the cytoplasm, the less of its protein can be translated.

Tubulin is a protein that polymerizes to form microtubules, a component of the cytoskeleton (see Chapter 4). When a large pool of free tubulin is available in the cytoplasm, there is no particular need for the cell to make more of it. Under these conditions, some tubulin molecules bind to tubulin mRNA. This binding makes the mRNA especially susceptible to breakdown, and less tubulin is made.

Translational and Posttranslational Control

Is the amount of a protein in a cell determined by the amount of its mRNA? Recently, a survey was made of the relationships between mRNA's and proteins in yeast cells. Dozens of genes were surveyed. For about a third of them, the relationship between mRNA and protein held: More of one led to more of the other. But for two-thirds of the proteins, there was no apparent relationship. Their concentration in the cell must be determined by factors acting after the mRNA is made.

Just as proteins can control the synthesis of mRNA by binding to DNA, they can also control the translation of mRNA by binding to mRNA in the cytoplasm. This mode of control is especially important in long-lived mRNA's. A cell must not continue to make proteins that it does not

74.20 Alternate Splicing Results in Different mRNA's and Proteins

In mammals, the protein tropomyosin is coded for by a gene that has 11 exons. Different tissues splice tropomyosin pre-mRNA differently, resulting in five different forms of the protein.

Exons

7

Introns

10 11

Different splicing patterns in different tissues result in a unique collection of exons in mRNA for each tissue.

Skeletal muscle: missing exon 2

Smooth muscle: missing exons 3 and 10

Fibroblast: missing exons 2, 3, and 10

Liver: missing exons 2, 3, 7, and 10

Brain: missing exons 2, 3, 10, and 11

Initially processed mRNA transcripts

:□:

:ezi

:nx

need. For example, mammalian cells respond to certain stimuli by making cyclins, which stimulate the events of the cell cycle. If the mRNA for a cyclin is still in the cytoplasm and available for translation long after the cyclin is needed, cyclin will be made and released inappropriately. Its presence might cause a target cell population to divide inappropriately, forming a tumor.

The translation of mRNA can be controlled

One way to control translation is by the capping mechanism on mRNA. As already noted, mRNA is capped at its 5' end by a modified guanosine molecule (see Figure 14.11). Messenger RNA's that have unmodified caps are not translated. For example, stored mRNA in the oocyte of the tobacco hornworm moth has the guanosine added to its 5' end, but the G is not modified. Hence, this stored mRNA is not translated. However, after fertilization, the cap is modified, allowing the mRNA to be translated to produce proteins needed for early embryogenesis.

Free iron ions (Fe^{2+}) within a mammalian cell are bound by a storage protein, ferritin. When iron is in excess, ferritin synthesis rises dramatically. Yet the amount of ferritin mRNA remains constant. The increase in ferritin synthesis is due to an increased rate of mRNA translation. When the iron level in the cell is low, a translational repressor protein binds to ferritin mRNA and prevents its translation by blocking its attachment to a ribosome. When iron levels rise, the excess iron binds to the repressor and alters its three-dimensional structure, causing it to detach from the mRNA, and translation of ferritin proceeds.

Translational control also acts in the synthesis of hemoglobin. As we described earlier, hemoglobin consists of four polypeptide chains and a nonprotein pigment, heme. If heme synthesis does not equal globin synthesis, some polypeptide chains stay free in the cell, waiting for a heme partner. Excess heme in the cell increases the rate of translation of globin mRNA by removing a block to the initiation of translation at the ribosome, helping to maintain the balance.

The proteasome controls the longevity of proteins after translation

We have considered how gene expression may be regulated by the control of transcription, RNA processing, and translation. However, the story does not end here, because most gene products—proteins—are modified after translation. Some of these changes are permanent, such as the addition of sugars (glycosylation), the addition of phosphate groups, or the removal of a signal sequence after a protein has crossed a membrane (see Figure 12.14).

An important way to regulate the action of a protein in a cell is to regulate its lifetime in the cell. Proteins involved in cell division (e.g., the cyclins) are hydrolyzed at just the right moment to time the sequence of events. Proteins identified for breakdown are often covalently linked to a 76-amino acid protein called ubiquitin (so called because it is ubiquitous, or widespread). The protein-ubiquitin complex then binds to a huge complex of several dozen polypeptide chains called a proteasome (Figure 14.21). The entryway to this "molecular chamber of doom" is a hollow cylinder, with ATPase activity, that cuts off the ubiquitin for recycling and unfolds its targeted protein victim. The protein then passes by three different proteases (thus the name of the complex) that digest it into small peptides and amino acids.

The cellular concentrations of many proteins are determined not by differential transcription of their genes, but by their degradation in proteasomes. For example, cyclins are degraded at just the right time during the cell cycle (see Figure 9.5). Transcription factors are broken down after they are used, lest the affected genes be always "on." Abnormal proteins are often targeted for destruction by a quality control mechanism. Human papillomavirus, which causes cervical cancer, targets the cell division inhibitory protein p53 for proteasomal degradation, so that unregulated cell division—and cancer—results.

Proteins targeted for breakdown are bound to ubiquitin, which "leads" them to the proteasome, a complex composed of many polypeptides.

f

A protein is targeted for breakdown.

QAn enzyme T ubiquitin

enzyme attaches

to the protein.

f

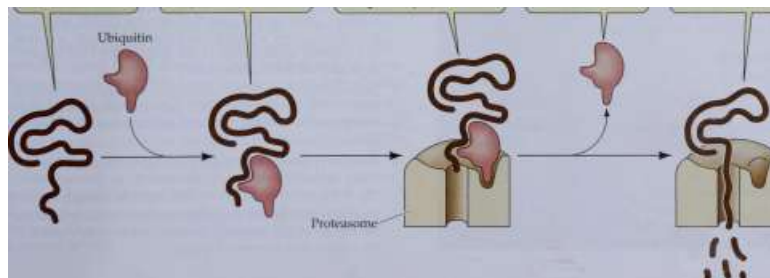
...and the complex is recognized by a proteasome.

f

Ubiquitin is released and recycled.

t

The complex hydrolyzes the target protein.



278 CHAPTER FOURTEEN

Chapter Summary

The Eukaryotic Genome

- ▶ Although eukaryotes have more DNA in their genomes than prokaryotes, in some cases there is no apparent relationship between genome size and organism complexity. Review Figure 14.1
- ▶ Unlike prokaryotic DNA, eukaryotic DNA is separated from the cytoplasm by being contained within a nucleus. The initial mRNA transcript of the DNA may be modified before it is exported from the cytoplasm. Review Figure 14.2
- ▶ The genome of the single-celled budding yeast contains genes for the same metabolic machinery as bacteria, with the addition of genes for protein targeting in the cell. Review Table 14.2
- ▶ The genome of the multicellular roundworm *Caenorhabditis elegans* contains genes required for intercellular interactions. Review Table 14.3
- ▶ The genome of the fruit fly has fewer genes than that of the roundworm. Many of its sequences are homologs of sequences on roundworm and mammalian genes.

Repetitive Sequences in the Eukaryotic Genome

- ▶ Highly repetitive DNA is present in up to millions of copies of short sequences. It is not transcribed. Its role is unknown.
- ▶ Telomeric DNA is found at the ends of chromosomes. Some telomeric DNA may be lost during each DNA replication, eventually leading to chromosome instability and cell death. The enzyme telomerase catalyzes the restoration of the lost telomeric DNA. Most somatic cells lack telomerase and thus have limited life spans. Review Figure 14.3
- ▶ Some moderately repetitive DNA sequences, such as those coding for rRNA's, are transcribed. Review Figure 14.4
- ▶ Some moderately repetitive DNA sequences are transposable, or able to move about the genome. Review Figure 14.5

The Structures of Protein-Coding Genes

- ▶ A typical protein-coding gene has noncoding internal sequences (introns) as well as flanking sequences that are involved in the machinery of transcription and translation. Review Figures 14.6, 14.8
- ▶ Nucleic acid hybridization is an important technique for analyzing eukaryotic genes. Review Figure 14.7

► Some eukaryotic genes form families of related genes that have similar sequences and code for similar proteins. These related proteins may be made at different times and in different tissues. Some sequences in gene families are pseudogenes, which code for nonfunctional mRNA's or proteins. Review Figure 14.9

► Differential expression of different genes in the (3-globin family ensures important physiological changes during human development. Review Figure 14.10

RNA Processing

► After transcription, the pre-mRNA is altered by the addition of a G cap at the 5' end and a poly A tail at the 3' end. Review Figure 14.11

► The introns are removed from the mRNA precursor by the spliceosome, a complex of RNA's and proteins. Review Figure 14.12

Transcriptional Control

► Eukaryotic gene expression can be controlled at the transcriptional, posttranscriptional, translational, and posttranslational levels. Review Figure 14.13

► The major method of control of eukaryotic gene expression is selective transcription, which results from specific proteins binding to regulatory regions on DNA.

► A series of transcription factors must bind to the promoter before RNA polymerase can bind. Whether RNA polymerase will initiate transcription also depends on the binding of regulatory proteins, activator proteins (which are bound by enhancers and stimulate transcription), and repressor proteins (which are bound by silencers and inhibit transcription). Review Figures 14.14,14.15

► The simultaneous control of widely separated genes is possible through proteins that bind to common sequences in their promoters. Review Figure 14.16

► The DNA-binding domains of most DNA-binding proteins have one of four structural motifs: helix-turn-helix, zinc finger, leucine zipper, or helix-loop-helix.

► Remodeling of chromatin occurs during transcription. Review Figure 14.17

► Heterochromatin is a condensed form of DNA that cannot be transcribed. It is found in the inactive X chromosome of female mammals. Review Figure 14.18

► The movement of a gene to a new location on a chromosome may alter its ability to be transcribed, as in the change from one mating type to another in yeast.

► Some genes become selectively amplified, and the extra copies result in increased transcription of their protein product. Review Figure 14.19

Posttranscriptional Control

► Because eukaryotic genes have several exons, alternate splicing can be used to produce different proteins. Review Figure 14.20

► The stability of mRNA in the cytoplasm can be regulated by the binding of proteins.

Translational and Posttranslational Control

► Translational repressors can inhibit the translation of mRNA.

► Proteasomes degrade proteins targeted for breakdown. Review Figure 14.21

For Discussion

1. In rats, a gene 1,440 bp long codes for an enzyme made up of 192 amino acid units. Discuss this apparent discrepancy. How long would the initial and final mRNA transcripts be?

2. The activity of the enzyme dihydrofolate reductase (DHFR) is high in some tumor cells. This activity makes the cells resistant to the anticancer drug methotrexate, which targets DHFR. Assuming that you had the complementary DNA for the gene that encodes DHFR, how would you show whether this increased activity was due to increased transcription of the single-copy DHFR gene or to amplification of the gene?

3. Describe the steps in the production of a mature, translatable mRNA from a eukaryotic gene that contains introns. Compare this to the situation in prokaryotes (see Chapter 13).

4. A certain protein-coding gene has three introns. How many different proteins can be made from alternate splicing of the pre-mRNA transcribed from this gene?

5. Most somatic cells in mammals do not express telomerase. Yet the germ line cells that produce gametes by meiosis do

express this enzyme. Explain.

15

Cell Signaling and Communication



J)) Carol had intended to complete the re-»*Ss port summarizing her semester's biology lab project long before it was due; it would, after all, count for significant points toward her grade. But between her other courses and a few interesting distractions, she kept putting off the report until "next week." Finally, at 9:30 the night before it was due, she sat down to create the 20-page report. By 11:00 she realized she still had hours of work to do. She made a frantic call to her lab partner, asking him to provide her with some data from one of their experiments. And Carol filled the coffee-brewing machine in her dorm room, knowing she would need a "caffeine jolt" to keep her awake so she could finish the job.

To understand how a "caffeine jolt" works, we must understand the pathways by which the body's cells respond to certain signals in their environment. The signals might be chemicals traveling between brain cells, or hormones produced in response to an outside event. There are three sequential processes involved in the cell's response to any signal. First, the signal binds to a receptor protein. Second, the binding of the signal causes a message to be conveyed to the cell's cytoplasm and amplified. Third, the cell changes its activity in response to the signal.

Caffeine acts in different ways in different tissues. First, a tired person's brain produces adenosine molecules that bind to specific receptor proteins, resulting in decreased brain activity and increased drowsiness. Caffeine's molecular structure is similar to that of adenosine, so it occupies the adenosine receptors without inhibiting brain cell function, and alertness is restored. Then, in the heart and liver, caffeine stimulates a pathway inside cells so that they do not need hormonal stimulation. In the heart, the result is an increased rate of beating; the liver is stimulated to release glucose into the bloodstream.

We begin this chapter with a discussion of signals that affect cells. As you will see, these range from chemicals such as hormones to physical entities such as light. Whatever the signal, it will affect a cell only if that cell has a receptor protein that binds to the signal. In addition to binding the signal, the receptor must somehow communicate to the rest of the cell that binding has occurred. This process of signal transduction often involves special small molecules

Multiple Signals at Work

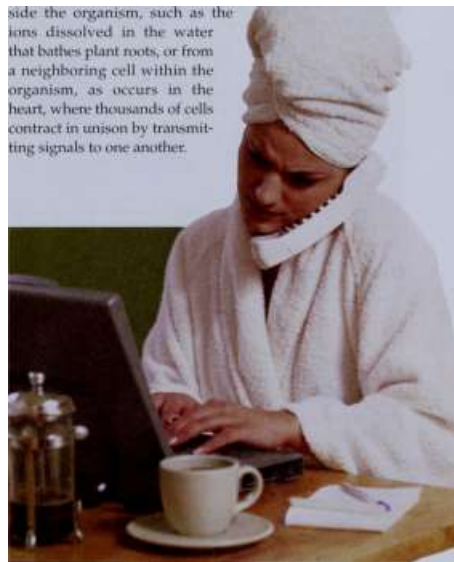
The computer, the telephone, and the coffee all send signals to the brain. All may be necessary for the successful completion of a research project.

called second messengers, which initiate a series of events that amplify the signal. As a result, the third phase—alteration of cell function—may involve many instances of the same event, such as the opening of an ion channel in the plasma membrane or increased transcription of a number of genes.

We close with a description of how cells communicate with one another directly through specialized channels in their adjacent plasma membranes.

Signals

Both prokaryotic and eukaryotic cells process information from their environment. This information can be in the form of a physical stimulus, such as the light reaching your eye as you read this book, or chemicals that bathe a cell, such as lactose in the medium surrounding *E. coli*. It may come from outside the organism, such as the ions dissolved in the water that bathes plant roots, or from a neighboring cell within the organism, as occurs in the heart, where thousands of cells contract in unison by transmitting signals to one another.



280 CHAPTER FIFTEEN

Of course, the mere presence of a signal does not mean that a cell will respond, just as you do not pay close attention to every sound in your environment as you study. To respond, the cell must have a specific receptor protein that can bind to the signal. In this section, we describe some of the signals different cells respond to and look at one model system of signal transduction.

Cells receive signals from the physical environment and from other cells

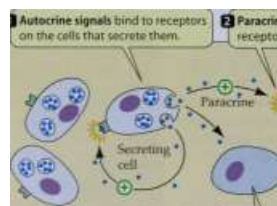
The physical environment is full of signals. Our sense organs allow us to respond to light, odors, touch, and sound. Bacteria and protists respond to even minute chemical changes in their environment. Plants respond to light as a signal. For example, at sunset, at night, or in the shade, not only the amount of sunlight but also the spectrum of light reaching the surface of Earth differs from that of full sunlight in the daytime. These variations are signals that affect plant growth and reproduction. Even magnetism can be a signal: Some bacteria and birds orient themselves to the Earth's magnetic poles, like a needle on a compass.

But a cell inside a large organism is far from the exterior environment. Instead, its environment is other cells and extracellular fluids. Cells receive their nutrients from, and

Local signals

I

Autocrine signals bind to receptors on the cells that secrete them.



Paracrine signals bind to receptors on nearby cells.

Receptor CO.



Target cell

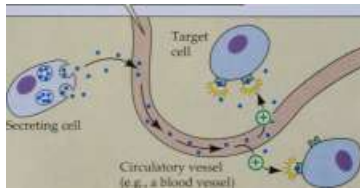
f- Not a target cell (no receptors)

Q Cells without receptors do not respond to a particular signal.

Distant signals

f

Circulating signals are transported by the circulation to bind to receptors on distant cells.



Secreting cell

Circulatory vessel (e.g., a blood vessel)

%

Target cell

7 5.7 Chemical Signaling Systems

A signal molecule can act on the same cell that produces it, or on a nearby cell. Most signals act on distant cells, to which they are transported by the organism's circulatory system.

Two signal molecules found in plants

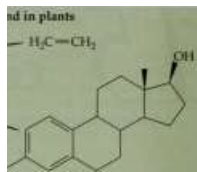
Ethylene is a gas that stimulates fruit to ripen



Brassinolide is a steroid that stimulates plant growth.

HO

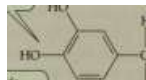
Two signal molecules found in animals



Epinephrine is a hormone that stimulates blood pressure and glycogen breakdown.

HO

Glucagon is a peptide that stimulates glucose synthesis in the liver.



CH₉

-N - \

H

H

OH

CH_q

+ H₃ N-

w' m - coo-

75.2 A Variety of Biological Signals

Many different kinds of molecules can serve as biological signals. The structure of glucagon is simplified so that a "bead" represents an amino acid, each about the size of the epinephrine molecule whose chemical formula is shown.

pass their wastes into, extracellular fluids. Cells also receive signals—mostly chemical signals—from their extracellular fluid environment. Most of these chemical signals come from other cells. In animal cells, they include hormones (Chapter 41), neurotransmitters (Chapter 44), and chemical messages from the immune system (Chapter 19). Cells also respond to chemical signals coming from the environment via the digestive and respiratory systems. And cells can respond to chemicals, such as CO₂ and H⁺, whose presence in the extracellular fluids results from the metabolic activities of other cells.

Inside a large organism, chemical signals reach a target cell by local diffusion or by circulation within the blood. Autocrine signals affect the cells that make them. Paracrine signals diffuse to nearby cells. Signals to distant cells usually travel through the circulatory system (Figure 15.1).

The biological signals cells receive are diverse (Figure 15.2). In each case, the cell must be able to receive or sense the signal and respond to it. Depending on the cell and the signal, the responses range from entering the cell division

cycle to heal a wound, to moving to a new location in the embryo to form a tissue, to releasing enzymes that digest food, to sending messages to the brain about the book you are reading. Clearly, signaling underlies a lot of biology. The whole process, from signal detection to final response, is called a signal transduction pathway.



75.3 A Model System for Signal Transduction

E.coli responds to an increase in solute concentration in its environment. The steps and systems illustrated here are found in all living organisms.

Environment

Receptor

The EnvZ membrane protein changes shape in response to the high solute concentration adding a phosphate to itself.

Transduction

Signaling involves a receptor, transduction, and effects

In Chapter 13, we saw that bacteria respond to changes in nutrients in their environment by altering their transcription of genes, as in the lac operon. In addition to responding to such changes, these same bacteria must be able to sense and respond to non-nutritive changes in their environment, such as changes in osmotic concentration. For example, if the solute concentration around E. coli rises far above that inside the cell, the law of diffusion tells us that water will diffuse out of the cell and solutes into the cell. Since the cell must maintain homeostasis in its cytoplasm, it must perceive and respond to this environmental change. The way in which this one-celled organism responds to such signals has much in common with signaling in more complex animals and plants (Figure 15.3).

The receptor protein in E. coli for osmotic changes is called EnvZ. It is a transmembrane protein that extends through the bacterium's plasma membrane into the space between the plasma membrane and a highly porous outer membrane that forms a complex with the cell wall. When the solute concentration of the extracellular environment rises, so does the concentration in the environment between the two membranes. This change in its aqueous medium causes the part of the receptor protein sticking into the intermembrane space to undergo a conformational change.

We saw in Chapter 6 that changing the tertiary structure of one part of a protein often leads to changes in distant parts of the protein. In the case of the bacterial EnvZ receptor, the conformational change in the intermembrane region of the protein is transmitted to the region that lies in the bacterium's cytoplasm. This change initiates the events of signal transduction. EnvZ becomes an active protein kinase, which catalyzes the addition of a phosphate group from ATP to one of EnvZ's own histidine residues. In other words, EnvZ phosphorylates itself.

The charged phosphate group added to the histidine causes the cytoplasmic tail of the EnvZ protein to change its shape again. It now binds to a second protein, OmpR,

Solutes enter the space between the two membranes through large pores in the outer membrane of E.coli.

Solute (signal)

on WmxSavm HfMMY///).

Intermembrane

space

Outer

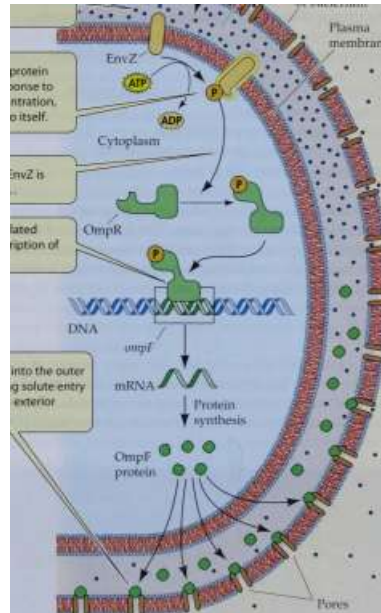
membrane/wall of bacterium

Plasma membrane

The phosphate from EnvZ is transferred to OmpR...

...and the phosphorylated OmpR initiates transcription of the ompF gene.

OmpF protein inserts into the outer membrane, preventing solute entry and keeping the cell's exterior osmotically balanced.



which takes the phosphate group from EnvZ. OmpR also changes its structure due to this phosphorylation. This change is a key event in signaling for three reasons. First, the signal on the outside of the cell has now been transduced to a protein totally within the cell's cytoplasm. Second, OmpR can do something, and that is to bind to a promoter on *E. coli* DNA adjacent to the DNA coding for the protein OmpF. This binding begins the final phase of this signaling pathway: the effect of the signal, which is an alteration in cell function. Third, the signal has been amplified. EnvZ can alter the structure of many OmpR molecules.

282 CHAPTER FIFTEEN

Phosphorylated OmpR has the correct three-dimensional structure to bind to DNA at the *ompF* promoter, resulting in an increase in transcription of that gene. Translation of *ompF* mRNA results in the production of OmpF protein, which is inserted into the outer membrane and prevents solutes from entering the intermembrane space. Thus the *E. coli* cell can go on behaving just as if the environment has a normal osmotic concentration.

It is important to highlight the major features of this prokaryotic system, as they will reappear in many other signal transduction systems in animals and plants:

- ▶ A receptor changes its conformation upon interacting with the signal.
- ▶ A conformational change exposes a protein kinase activity.
- ▶ Phosphorylation alters the function of a protein.
- ▶ The signal is amplified.
- ▶ Transcription factors are activated.
- ▶ Altered synthesis of specific proteins occurs.
- ▶ Protein action alters cell activity.

Signal transduction pathways featuring these seven activities occur in all types of organisms. The emergence of these activities was an important event in the evolution of cellular life, as they allowed the organism to react to and survive in a rapidly changing environment.

Receptors

While a given cell is bombarded with many signals, it responds to only a few of them. The reason for this is that any given cell makes receptors for only some signals. Which cells make which receptors is genetically determined: If a

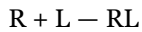
cell transcribes the gene encoding a particular receptor and the resulting mRNA is translated, the cell will have that receptor. A receptor protein binds to a signal in much the same way as an enzyme binds to a substrate or a membrane transport protein binds to the molecule it is transporting.

Receptors have specific binding sites for their signals

A signaling molecule, usually called a ligand, fits into a site on its receptor much like a substrate fits into the active site of an enzyme (Figure 15.4). Whether the receptor protrudes from the plasma membrane surface or is located in the cytoplasm, the result of ligand binding is the same: The receptor protein changes its three-dimensional structure and initiates a cellular response. The ligand does not contribute further to this response. In fact, the ligand usually is not metabolized into useful

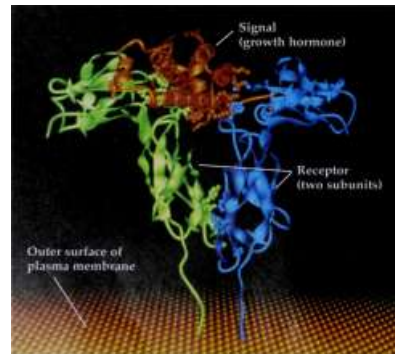
products. Its role is purely to "knock on the door." This is in sharp contrast to enzyme-substrate interactions, in which the whole purpose is to change the substrate into a useful product.

Receptors bind to their ligands according to chemistry's law of mass action:



This means that the binding is reversible, although for most ligand/receptor complexes, the equilibrium point is far to the right—that is, favoring binding. Release of the ligand is important because if it does not happen, the receptor will be continuously stimulated.

Just as with enzymes, inhibitors can bind to the ligand site on a receptor protein. Both natural and artificial inhibitors of receptor binding are important in medicine.



75.4 A Signal Bound to Its Receptor

Only the extracellular regions of the human growth hormone receptor are shown.

Outside of cell

Nonpolar signal

Membrane-bound receptor

Plasma membrane

Polar

.signal

y



A

A signal that is polar and/or large cannot diffuse through the plasma membrane. Its receptor is embedded in the membrane.

A nonpolar signal can diffuse directly across the bilayer of the plasma membrane to encounter its receptor in the cytoplasm.

Cytoplasm

75.5 Two Locations for Receptors

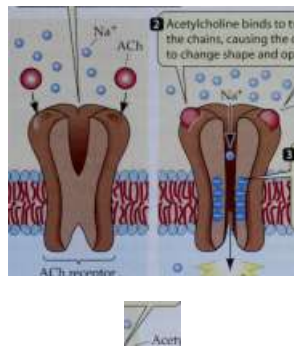
Receptors can be located on the plasma membrane or in the cytoplasm of the cell.

■ 1.

I]The acetylcholine receptor, a gated ion channel, is made up of five polypeptide subunits.

Q Acetylcholine binds to two of the chains, causing the channel to change shape and open.

oo°o°



Acetylcholine (ACh)

ACh receptor Cytoplasm



The channel is lined with negatively charged amino acids, allowing Na^+ to flow into the cell.

Na^+ buildup in cells leads to muscle contraction.

15.6 An Ion Channel Receptor

The acetylcholine receptor is a channel for sodium ions that resembles a gate. The gate opens when its ligand, acetylcholine, binds to it, allowing Na^+ to flow into the cell.

There are several types of receptors

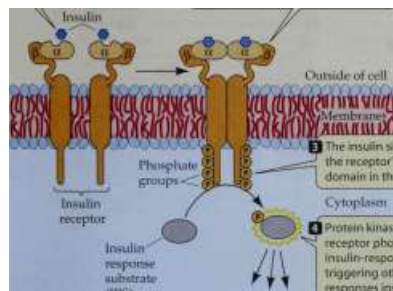
A major division among receptors is in their cellular location, which largely depends on the nature of their ligands. The chemistry of signals is quite variable, but they can be divided into two classes: those that are nonpolar, and can cross the plasma membrane and enter the cell, and those that are large or polar, and cannot cross the membrane (Figure 15.5). Estrogen, for example, is a steroid and can easily diffuse across the plasma membrane and enter the cell; it binds to a receptor inside the cytoplasm. Insulin, on the other hand, is



The α subunit binds insulin (the signal).

f

The β subunit transmits a signal from bound insulin to the cytoplasm.



a protein hormone that cannot diffuse through the plasma membrane; instead, it binds to a receptor that is a transmembrane protein with an extracellular binding region.

In more complex eukaryotes, there are three well-studied types of receptors on plasma membranes: ion channels, protein kinases, and G protein-linked receptors.

ion channels. In the plasma membranes of many types of cells, there are channel proteins that can be open or closed. These ion channels act as "gates," allowing ions such as Na^+ , K^+ , Ca^{2+} , or Cl^- to enter or leave the cell. The gate-opening mechanism is an alteration in the three-dimensional structure of the channel protein upon ligand binding. Each ion channel has its own signal. These signals include sensory stimuli, such as light and sound, voltage differences across the plasma membrane, and chemical ligands such as small molecules and hormones.

An example of a gated ion channel is the acetylcholine receptor (Figure 15.6). This receptor is located at the plasma membranes of vertebrate skeletal muscle cells and binds the ligand acetylcholine, which is released from nerve cells. When two molecules of acetylcholine bind to the receptor, it opens for about a thousandth of a second. This is enough time for Na^+ , which is more concentrated outside the cell than inside, to rush into the cell. The change in Na^+ concentration in the cell ultimately results in muscle contraction. Right after the channel opens, the ligand is released from the receptor and then degraded. This makes the receptor (and the cell) responsive to the next signal, so that the muscle can contract again.

protein kinases. Like the activated EnvZ protein of *E. coli*, some eukaryotic receptor proteins become kinases when they are activated: That is, they catalyze the transfer of a phosphate group from ATP to a protein. The targets for the protein kinase activity are both the receptor itself and cytoplasmic molecules, which alter their shape and then act to change the cell's activities. While histidine is phosphorylated by EnvZ in *E. coli*, the amino acid targeted by the protein kinase receptors of animal cells is usually tyrosine. In plants, either serine or threonine is phosphorylated.

Insulin is a protein hormone made by the mammalian pancreas. The receptor for insulin is a protein consisting of two copies each of two different polypeptide subunits (Figure 15.7). As with acetylcholine, two molecules of insulin must bind to the receptor. After binding insulin on its extracellular surface, the receptor changes

The insulin signal activates the receptor's protein kinase domain in the cytoplasm.

Insulin receptor

Insulin response substrate (IRS)

Cellular responses

Protein kinases from the receptor phosphorylate insulin-response substrates, triggering other chemical responses inside the cell.

75.7 A Protein Kinase Receptor

The mammalian hormone insulin does not enter the cell, but is bound by a membrane receptor protein with four subunits (two α and two β). The β subunits transmit a signal that changes the cytoplasmic end of the receptor protein, activating a protein kinase domain and triggering further responses by the cell, eventually resulting in the transport of glucose across the membrane.

284 CHAPTER FIFTEEN

it-- shape to expose a cytoplasmic protein kinase active site. Like the EnvZ receptor described above, the insulin receptor self-phosphorylates. Then, as a protein kinase signal, it targets certain cytoplasmic proteins, appropriately called insulin response substrates. These proteins then initiate many cell responses, including the insertion of glucose transporters into the plasma membrane.

G protein-linked receptors. A third category of eukaryotic plasma membrane receptor is the seven-spanning G protein-linked receptors. This long name identifies a fascinating group of receptors, all of which are composed of a single protein with seven regions that pass through the lipid bilayer, separated by short loops that extend either outside or inside the cell. Ligand binding on the extracellular side changes the shape of the receptor's cytoplasmic region, opening up a binding site for a mobile membrane protein.

This membrane protein, known as a G protein, has two important binding sites: one for the G protein-linked receptor, and the other for the nucleotide GDP/GTP (Figure 15.8). G proteins have several polypeptide subunits. When the G protein binds to the activated receptor, it also binds GTP to one of its subunits. At the same time, the ligand is released from the extracellular side of the receptor. The GTP-bound subunit of the G protein now separates from the parent G protein, diffusing in the plane of the lipid bilayer until it encounters an effector protein to which it can bind. Effector proteins are what their name implies: They cause an effect. The binding of the GTP-bearing G protein subunit activates the effector—which may be an ion channel or an enzyme—thereby causing changes in cell function.

After binding to the effector protein, the GTP on the G protein is hydrolyzed to GDP. The now inactive G protein subunit separates from the effector protein. The G protein subunit must form a complex with another subunit before binding to yet another activated receptor. When this activated receptor is bound, the G protein exchanges its GDP for GTP, and the cycle begins again.

By means of their diffusing subunits, G proteins can either activate or inhibit an effector. An example of an activating response involves the receptor for epinephrine (adrenaline), the famous "fight-or-flight" hormone made by the adrenal gland in response to stress or heavy exercise. In heart muscle, this hormone binds to its G protein-linked receptor, causing a G protein to become activated. The GTP-bound subunit then activates a membrane-bound enzyme to produce a small molecule, cyclic AMP (see below), which has many effects on the cell, including glucose mobilization for energy and muscle contraction.

An example of G protein-mediated inhibition occurs when the same hormone, epinephrine, binds to its receptor in the smooth muscle cells surrounding blood vessels lining the digestive tract. Again, the epinephrine-bound receptor changes its shape and binds a G protein, which then binds GTP, and the G protein subunit with its GTP binds to the target enzyme. But in this case, the enzyme is inhibited instead of being activated. As a result, the muscles relax, and the blood vessel diameter increases, allowing more nutrients to be carried away from the digestive system to the rest of the body. Thus the same signal and initial signaling mechanism can have different consequences in different cells, depending on the nature of the responding cell.

cytoplasmic receptors. Not all signals act at the plasma membrane. Some signals diffuse across the lipid bilayer of the plasma membrane and enter the cytoplasm. In these cases, the receptor protein lies inside the cytoplasm. Steroid hormones in animals, for example, enter the cytoplasm and bind to steroid hormone receptors. Binding to the ligand

Binding of an extracellular signal—in this case, a hormone—causes the activation of a seven-segmented G protein. The G protein then activates an enzyme that catalyzes a reaction in the cytoplasm, amplifying production of the product. The figure is a generalized diagram that could apply to any of the large family of G proteins and the signals they react with.



Q The actions of several membrane-associated proteins are required to convert the signal from a hormone to an amplified response in the cell.

t Hormc signal

Hormone binding provides a signal that activates the G protein.

Signal (hormone)

Outside * of cell fcX

H <

Q Part of the activated G protein activates an enzyme that converts thousands of reactants to products, thus amplifying the action of a single signal molecule.

_A

Receptor binds hormone

Inactive enzyme

Active enzyme

Cytoplasm

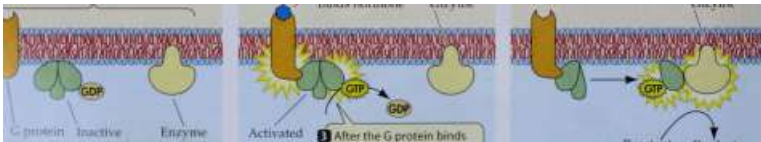
G protein Inactive ptor G protein

Enzyme molecule

Activated G protein

I After the G protein binds the receptor, GDP is exchanged for GTP, completing the activation.

Reactant Product



Amplification

Signal (Cortisol)

Outside of cell



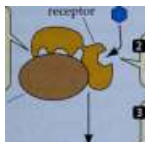
Cytoplasm

Q The chaperone protein causes the receptor to fold, so it cannot enter the nucleus.

Chaperone protein



Cortisol Y receptor





Nucleus



Cortisol enters the cytoplasm and binds to the receptor...

...causing the receptor to change

d shape and release the chaperone.

The receptor is now in a shape that can enter the nucleus.

^—5

15.9 A Cytoplasmic Receptor

The receptor for Cortisol is bound to a chaperone protein. Binding of the signal (which diffuses directly through the membrane) releases the chaperone and allows the receptor protein to enter the cell's nucleus, where it functions as a transcription factor.

(a) Direct transduction

Outside of cell

Signal

(^A

t

A signal binds to a receptor protein...

causes the receptor to change its shape so that it can enter the cell nucleus, where it acts as a transcription factor (Figure 15.9). But this general view is somewhat simplified. The receptor for the hormone Cortisol, for example, is bound to a chaperone protein, which blocks it from entering the nucleus. Binding of the hormone causes the receptor to change its shape so that the chaperone is released. This allows the receptor, which is a transcription factor, to fold into an appropriate configuration for entering the nucleus and initiating transcription.

Transducers

As previously mentioned, the same signal may produce different responses in different tissues. Acetylcholine, for example, can bind to receptors on skeletal muscle cells, where it stimulates muscle contraction, but on heart muscle cells, it slows contraction. These different responses to the same ligand/receptor complex are mediated by the events of signal transduction. These events, which are critical to the cell's response, may be either direct or indirect.

Direct transduction is a property of the receptor itself and occurs at the plasma membrane. In indirect transduction, which is more common, another molecule, termed a second messenger, mediates the interaction between receptor binding and cellular reaction. In neither case is transduction a single event. Rather, the signal initiates a cascade of events, in which proteins interact with other proteins until the final responses are achieved (Figure 15.10).

15.10 Direct and Indirect Signal Transduction

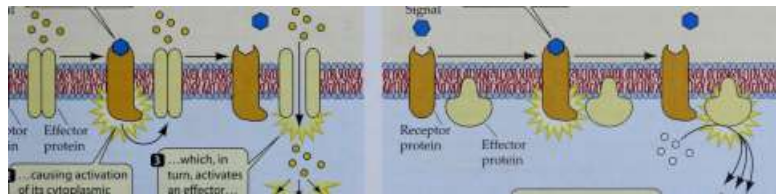
(a) All the events of direct transduction occur on the plasma membrane, at or near the receptor protein, (b) In indirect transduction, the binding of the signal to the receptor triggers formation of a "second messenger" molecule that works in the cytoplasm. It is the second messenger that sets off the necessary biochemical reactions.

(b) Indirect transduction

Signal

f

A signal binds to a receptor protein...



Receptor Effector protein protein

Q ...causing activation of its cytoplasmic domain...

S) ...which, in turn, activates an effector.

Q ...initiating transport into or out of the cell.



f

...leading to formation of a second messenger...



Cytoplasm

f

...that amplifies the signal.



•.V

•/|\

Cellular responses

286 CHAPTER FIFTEEN

Protein kinase cascades amplify a response to receptor binding

We have seen that when a signal binds to a protein kinase receptor, the receptor changes its structure to expose a protein kinase active site, which catalyzes the phosphorylation of target proteins. This process is an example of direct signal transduction. Protein kinase receptors are important in binding ligands that stimulate cell division in both plants and animals. In Chapter 9, we described growth factors that were external inducers of the cell cycle. These factors stimulate cell division by binding to protein kinase receptors.

The complete signal transduction pathway that occurs after a protein kinase receptor is activated was worked out from studies on a cell that went wrong. Many human bladder cancers contain an abnormal protein called Ras (so named because it was first isolated from a rat sarcoma tumor). Investigations of these bladder cancers showed that the Ras protein was a G protein, but was always active because it was permanently bound to GTP. So the abnormal Ras protein caused continuous tumor cell division. If the cancer cells' Ras protein was inhibited, the tumor cells stopped dividing. This discovery has led to a major effort to develop specific Ras inhibitors for cancer treatment.

What does Ras do in normal, noncancerous cells? When scientists treated cells in a laboratory dish with both a Ras inhibitor and a growth factor, the expected cell division did

not occur. Since growth factor binding is the first event in stimulating cell division, these results meant that Ras, like other G proteins, must be an intermediary between signal (growth factor) and response (cell division). After this discovery, the challenge was to work out what the activated growth factor receptor did to Ras, and what Ras did to stimulate further events in signal transduction.

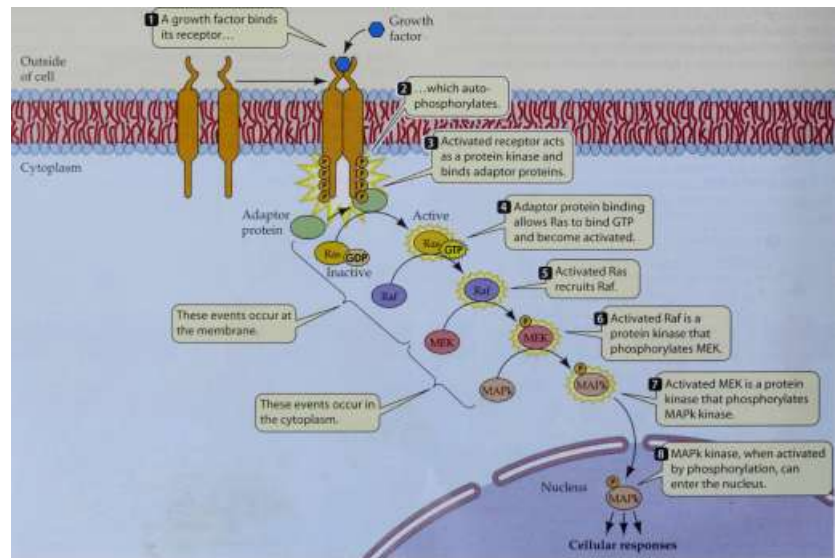
This signaling pathway has been worked out, and it is an excellent example of a more general phenomenon, a protein kinase cascade (Figure 15.11). Such cascades are key to the external regulation of many cellular activities. Indeed, as we saw in Chapter 14, the eukaryotic genome codes for hundreds, even thousands, of such kinases. The unbound receptors for growth factors exist in the plasma membrane as separate polypeptide chains (subunits). When the growth factor signal binds to a subunit, it associates with another subunit to form a dimer, which changes shape to expose a protein kinase active site. The kinase activity sets off a series of events, activating several other protein ki-

75.7 7 A Protein Kinase Cascade

In a protein kinase cascade, a series of proteins becomes sequentially activated. In this example, the growth factor receptor protein stimulates the G protein Ras, which mediates a cascading series of reactions. The final product of the cascade, MAP kinase (MAPk), enters the nucleus and causes changes in transcription. Inactive forms of the proteins are on the left, activated forms are on the right.

Growth factor •

Outside of cell



nases in turn. The final phosphorylated, activated protein— MAP kinase—moves into the nucleus and phosphorylates target proteins necessary for cell division.

Is the protein kinase cascade pathway universal in eu-karyotes? Genome sequencing of the plant Arabidopsis has revealed proteins with strong homologies to many of the proteins in the mammalian pathway. A number of proteins that resemble tyrosine kinase receptors are also present. There is even a Ras-like protein. The functions of this pathway in Arabidopsis are under investigation.

Protein kinase cascades are very useful for signal transduction for three reasons:

- At each step in the cascade of events, the signal is amplified. Because each newly activated protein kinase is an enzyme, each can catalyze the phosphorylation of many target proteins.
- The information from the signal that was originally at the plasma membrane is communicated to the nucleus.
- The multitude of steps provides some specificity to the process. As we have seen with epinephrine, signal binding and receptor activation do not result in the same response in all cells. Different target proteins at every step in the cascade can provide variability of response.

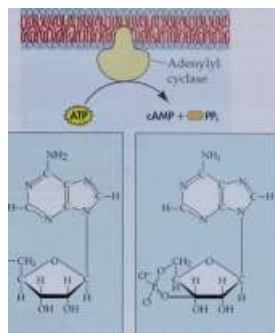
Phosphate groups

O O o ll II li

"O – P–O–P–O– P – O-CH,

I I I

o- o- o- c; h



AN—KA

OH OH

ATP

Cyclic AMP (cAMP)

75. 12 The Formation of Cyclic AMP

The formation of cAMP from ATP is catalyzed by adenylyl cyclase, an enzyme that is activated by G proteins.

Cyclic AMP is a common second messenger

As we have seen, protein kinase receptors stimulate the protein kinase cascade right at the plasma membrane. However, the stimulation of events in the cell is more often indirect. In a series of clever experiments, Earl Sutherland, Edwin Krebs, and Edmond Fischer showed that in many cases, there is a small, water-soluble chemical messenger between the membrane receptor and cytoplasmic events. These researchers were investigating the activation of the liver enzyme phosphorylase by the hormone epinephrine. Phosphorylase catalyzes the breakdown of glycogen stored in the liver so that carbohydrate can be released to the blood to fuel the fight-or-flight response.

The researchers found that phosphorylase could be activated in liver cells that had been broken open, but only if the entire cell contents, including the plasma membrane, were present. Epinephrine had bound to the plasma membrane, and phosphorylase was present in the cytoplasm. So they tried the steps of this experiment in sequence. First, they incubated membranes of broken liver cells with epinephrine. Then they removed the membranes, but kept the solution in which the membranes had been bathed. When they added this solution to the cytoplasm, the phosphorylase enzyme present became activated! Hormone binding to the membrane receptor had caused the production of a small, water-soluble molecule that then diffused to the cytoplasm, where it activated the enzyme.

This small molecule was identified as cyclic AMP (cAMP), which we also encountered in the lac operon regulatory system in *E. coli*, where cAMP was working as a second messenger. Second messengers are substances re-

leased into the cytoplasm after the first messenger—the signal—binds its receptor.

In contrast to the uniqueness of receptor binding, second messengers affect many processes in the cell, and allow a cell to respond to a single event at the plasma membrane with many events inside the cell. Like the kinase cascade, second messengers amplify the signal—a single epinephrine molecule leads to the production of several dozen molecules of cAMP, which then activate many enzyme targets.

Adenylyl cyclase, the enzyme that catalyzes the formation of cAMP from ATP, is located on the cytoplasmic surface of the plasma membrane of target cells (Figure 15.12). Usually, it is activated by the binding of G proteins, themselves activated by receptors. Second messengers do not have enzymatic activity; rather, they act as cofactors or allosteric regulators of target proteins. In the case of cAMP, there are two major target types. In many kinds of sensory cells, cAMP binds to ion channels to cause them to open. A second major target type is cytoplasmic. Cyclic AMP binds to an enzyme such as a protein kinase, whose active site gets exposed as a result. The sequential activation of yet another kinase ensues, leading to the final effects in the cell.

Two second messengers are derived from lipids

Membrane phospholipids are involved in signal transduction in addition to their roles as structural components of the plasma membrane. When certain phospholipids are hydrolyzed into their component parts (see Figure 3.21) by enzymes called phospholipases, second messengers are formed. The best-studied of these come from hydrolysis of the lipid phosphatidylinositol-bisphosphate (PTI), which

288 CHAPTER FIFTEEN

Hormone v. Outside

of cell



Cytoplasm



The receptor binds the hormone. J

Receptor PTI

Phospholipase C DAG Plasma

membrane

/

8WwSK^

i-l/. it Ml/ irilVVlr.

fotspz:



Q DAG, along with the Ca^{2+} produced by IP_3 activity, activates protein kinase C(PKC).

f

Activated G protein dissociates and activates phospholipase C.



IP_3 , *P'

Q The activated enzyme produces the second J messengers DAG and

IP_3 , from PTI.



f

IP_3 opens Ca^{2+} channel.

Lumen of smooth endoplasmic reticulum

75.73 The IP_3 and DAG Second Messenger System

Phospholipase C hydrolyzes the lipid phosphatidyl inositol-bis-phosphate (PTI) into its components IP_3 and DAG, both of which are second messengers. IP_3 and DAG act separately but in concert, ultimately producing a wide range of responses in the cell.

has two fatty acid chains (diacylglycerol, or DAG) embedded in the plasma membrane, and a hydrophilic inositol group (inositol triphosphate, or IP_3) projecting into the cytoplasm. There are over two dozen signals whose actions are mediated by the products of PTI hydrolysis. Once again, the receptors involved are often linked to G proteins. The activated G proteins diffuse through the plasma membrane and activate an enzyme, phospholipase C. This enzyme cleaves off the IP_3 from PTI, leaving the glycerol and the two attached fatty acids (DAG) embedded in the lipid bilayer:

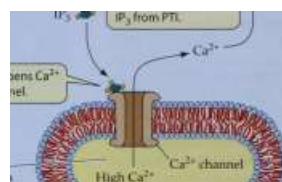
Phospholipase C

IP_3 + DAG

IP_3 and DAG are both second messengers and have different modes of action that build on each other (Figure 15.13). DAG activates a membrane-bound enzyme, protein kinase C (PKC), in much the same way that cAMP activates protein kinase A. PKC is dependent on Ca^{2+} (hence the "C"), and this is where IP_3 comes in. IP_3 is charged and diffuses through the cytoplasm to the endoplasmic reticulum, where it causes the release of Ca^{2+} into the cytoplasm. There, in combination with DAG, the Ca^{2+} causes PKC to become active. PKC then phosphorylates a wide variety of proteins, leading to the ultimate response of the cell (Figure 15.13).

Calcium ions are involved in many transduction pathways

Calcium ions can also act as a second messenger. They are scarce in most cells, with a cytoplasmic concentration of only about 0.1 μM , while the concentrations of Ca^{2+} outside

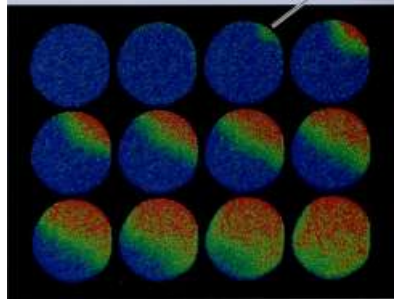


The phosphorylation enzymes and other proteins occurs in response pathways.

the cell and within the ER are usually much higher. This difference is maintained by active transport proteins at the plasma and ER membranes that pump the ion out of the cytoplasm. In contrast to cAMP and the lipid second messengers, the level of intracellular Ca^{2+} cannot be increased by making more of it. Instead, the opening and closing of channels and the action of membrane pumps regulate levels of the ion in a cellular compartment.

There are many signals that can cause Ca^{2+} channels to open, including IP₃ (see the previous section) and the entry of a sperm into an egg cell (Figure 15.14). Whatever the signal, the open channels result in a dramatic increase in cyto-

Sperm entry point



75. 14 Calcium Ions as an Intracellular Messenger

The concentration of Ca^{2+} can be measured by a dye that fluoresces and turns red when it binds the ion. Here, photographed at 5-second intervals, fertilization causes a wave of Ca^{2+} to pass through the egg of a starfish. The message that fertilization is complete and development can begin is thus delivered.

plasmic Ca^{2+} concentration, up to a hundredfold within a fraction of a second. As we saw earlier, this ion activates protein kinase C. In addition, Ca^{2+} controls other ion channels and stimulates secretion by exocytosis.

A distinctive aspect of Ca^{2+} signaling is that the ion can stimulate its own release from intracellular stores. For example, in some plant leaf cells, the hormone abscisic acid binds to gated Ca^{2+} channels and opens them, causing the ion to rush into the cells. This influx is not enough to trigger the cell's response, however. The ion binds to Ca^{2+} channels in the endoplasmic reticulum and vacuolar membranes, causing those organelles to release their Ca^{2+} stores as well.

In some cases, Ca^{2+} ions act via a calcium-binding protein called calmodulin, and it is the Ca^{2+} -calmodulin complex that performs cellular functions by binding to target proteins. Calmodulin, which is present in many cells, has four binding sites for Ca^{2+} . When the cytoplasmic Ca^{2+} concentration is low, calmodulin does not bind enough of it to become activated. But when the cell is stimulated by a signal and the Ca^{2+} level rises, all four binding sites are filled. Then the calmodulin changes shape and binds to a number of cellular targets, activating them in turn. One such target is a protein kinase in smooth muscle cells that phosphorylates the muscle protein myosin, initiating muscle contraction.

Nitric oxide is a gas that can act as a second messenger

Pharmacologist Robert Furchgott, at the State University of New York in Brooklyn, was investigating how acetylcholine causes smooth muscles lining blood vessels to relax, thus allowing more blood to flow to certain organs. Acetylcholine appeared to stimulate the IP₃ signal transduction system to produce an influx of Ca^{2+} , which led to an increase in the level of an unusual second messenger, cyclic GMP (cGMP). This nucleotide bound to a protein kinase, which then stimulated a kinase cascade leading to muscle relaxation. So far, the pathway seemed straightforward.

But while this pathway seemed to work in intact animals, it did not work on isolated strips of artery tissue. When Furchgott switched to tubular sections of artery, however, signal transduction did occur. There turned out to be a crucial difference between these two tissue preparations: In the strips, the delicate inner layer of cells that lines the blood vessel had been lost. Furchgott hypothesized that this layer, the endothelium, was making something that diffused into the muscle cells and was needed for their response to acetylcholine. The substance was not easy to isolate. It seemed to break down quickly, with a half-life (the time in which half of it disappeared) of 5 seconds in living tissues. It turned out to be a gas, nitric oxide (NO), that had always been thought of as a toxic air pollutant!

In the body, NO is made via an enzyme, NO synthase. This enzyme is activated by Ca^{2+} , which enters the endothelial cell through a channel opened by IP₃, released after acetylcholine binds to its receptor. The NO formed is chem-

ically very unstable and although it diffuses readily, it does not get too far. Conveniently, the endothelial cells are close to the smooth muscle cells, where NO acts as a second messenger, stimulating the formation of cGMP (Figure 15.15).

The spectacular discovery of NO as a second messenger explained the action of nitroglycerin, a drug that has been used for over a century to treat angina, the chest pain caused by insufficient blood flow to the heart. Nitroglycerin releases NO, which results in relaxation of the blood vessels and increased blood flow.

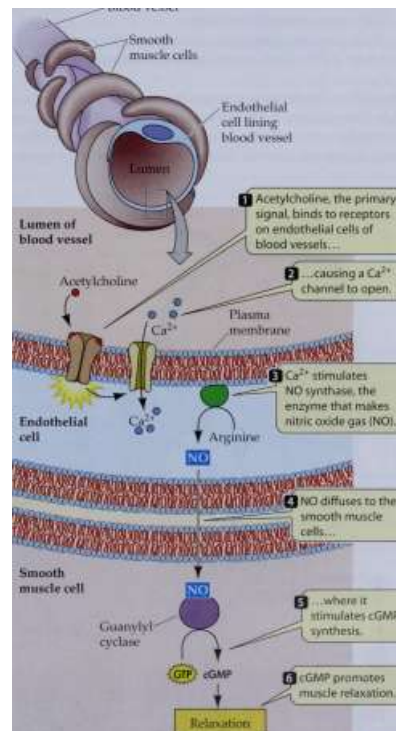
Blood vessel

7 Smooth muscle cells

Endothelial cell lining blood vessel

Acetylcholine, the primary signal, binds to receptors on endothelial cells of blood vessels...

...causing a Ca^{2+} channel to open.



cGMP promotes muscle relaxation.

7.5.7.5 Nitric Oxide as a Second Messenger

Nitric oxide (NO) is an unstable gas, which nevertheless serves as a second messenger between a signal, acetylcholine, and its effect, the relaxation of smooth muscles. The endothelial tissue of blood vessels is a crucial intermediary in this communication between three types of tissue.

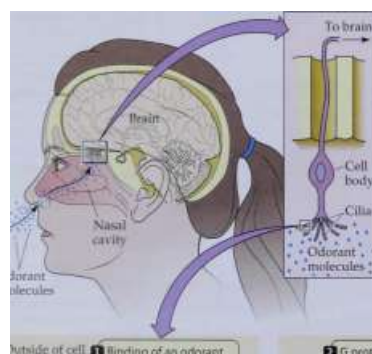
290 CHAPTER FIFTEEN

Signal transduction is highly regulated

There are several ways in which cells can regulate the activity of a signal transduction mechanism. The concentration of NO, which breaks down quickly, can be regulated only by how much of it is made. The level of Ca^{2+} , on the other hand, is determined by both membrane pumps and ion channels. For protein kinase cascades, G proteins, and cAMP, there are enzymes that convert the activated form back to its inactivated precursor:

- Protein phosphatases remove the phosphate groups from phosphorylated proteins.
- GTPases convert the GTP on an active G protein back to GDP, inactivating the protein.
- cAMP phosphodiesterase converts cAMP into its precursor, AMP, which has no second messenger activity.

These three inactivation systems are themselves under controls. For example, the major protein phosphatase can be inhibited by a protein whose activity is determined by phosphorylation by protein kinase A, which itself is under



Odorant molecules

Outside of cell o Binding of an odorant to its receptor activates ^Odorant [a G protein, molecule

control of cAMP So the cAMP pathway can intersect with the protein kinase cascade. On the other hand, some cAMP phosphodiesterases are stimulated by Ca^{2+} , thus showing an interaction between these two signaling pathways. The caffeine in coffee acts as a stimulant in part because it inhibits cAMP phosphodiesterase.

Effects

We have seen that the binding of a signal to its receptor initiates the response of a cell to an environmental signal, and how the direct or indirect transduction of this signal to the cytoplasm of the cell amplifies the stimulus. In this section, we consider the third and final step in the process, the actual effects of the signal on cell function. These effects are primarily the opening of membrane channels, changes in the activities of enzymes, and differential gene transcription.

Membrane channels are opened

The opening of ion channels is of great importance when the nervous system responds to a signal. Sensory nerve cells of the sense organs, for example, become stimulated through the opening of ion channels. We will focus here on one such signal transduction pathway, that for the sense of smell (Figure 15.16).

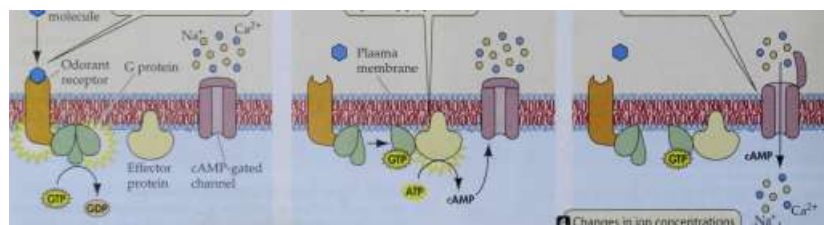
The sense of smell is well developed in mammals, some of which have an amazing 1,000 genes for odorant receptors, the largest gene family known. Each of the thousands of nerve cells in the nose expresses one of these receptors. The identification of which chemical signal, or odorant, activates which receptor is just getting under way.

75.76 A Signal Transduction Pathway Leads to the Opening of Membrane Channels

In the signal transduction pathway for the sense of smell, the final effect is the opening of Na^+ channels. The resulting influx of Na^+ stimulates the transmission of a scent message to a specific region of the brain.

G protein activates the synthesis of cAMP by adenylyl cyclase.

cAMP activates the opening of ion channels.



Cytoplasm

Changes in ion concentrations inside the cell send a signal to a specific area of the brain, which perceives the signal as a scent.

I.

Signal to brain

Outside of cell

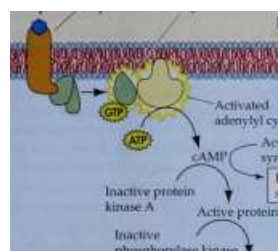
Epinephrine

Epinephrine receptor



">

Activated G protein



Phosphorylation, induced by epinephrine binding, inactivates glycogen synthase, preventing glucose from being stored as glycogen.

adenylyl cyclase

Active glycogen synthase

Inactive protein kinase A

Inactive glycogen synthase

Active protein kinase A

Inactive phosphorylase kinase

Active phosphorylase kinase Inactive glycogen phosphorylase

Q Phosphorylation activates glycogen phosphorylase, releasing stored glucose molecules from glycogen.

Active glycogen phosphorylase

Glycogen

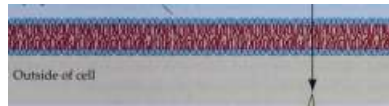
Glucose-1-phosphate

Cytoplasm

Plasma membrane

\

Glucose



Outside of cell

f

Release of glucose triggers "fight or flight" response.

When an odorant molecule binds to its receptor, a G protein becomes activated, which in turn activates adenylyl cyclase to form the second messenger cAMP. This molecule then binds to an ion channel, causing it to open. The resulting influx of Na^+ causes the nerve cell to become stimulated so it sends a signal back to the brain that a particular odor is present.

Enzyme activities are changed

Proteins will change their shape, and their functioning, if they are modified either covalently or noncovalently. We have seen examples of both types of modification in signal transduction. Protein kinases add phosphate groups to a target protein, and the covalent change alters the protein's shape. Cyclic AMP binds to target proteins allosterically, and this noncovalent interaction changes the protein's shape. In both cases, previously inaccessible active sites are exposed, and the target protein goes on to perform a cellular role.

[fil^{75.77} A Cascade of Reactions Leads to Altered Enzyme Activity

Liver cells respond to epinephrine by activating G proteins, which in turn activate cAMP synthesis. The second messenger initiates a series of kinase reactions. The cascade both inhibits the continued storage of glucose molecules and stimulates the release of previously stored glucose.

The G protein-mediated protein kinase cascade stimulated by epinephrine in liver cells results in the phosphorylation of two key enzymes in glycogen metabolism (Figure 15.17). One of them, glycogen synthase, catalyzes the joining of glucose molecules to synthesize the energy-storing molecule glycogen, but it is inactivated by phosphorylation. Thus the epinephrine signal prevents glucose from being stored in glycogen. On the other hand, phosphorylase kinase becomes activated when a phosphate group is added to it, and goes on to stimulate a protein kinase cascade that ultimately leads to the activation by phosphorylation of glycogen phosphorylase, the other key enzyme in glucose metabolism. This enzyme liberates glucose molecules from glycogen. Thus the same signaling pathway inhibits the storage of glucose as glycogen (by inhibiting glycogen synthase) and promotes the release of glucose through glycogen breakdown (by activating glycogen phosphorylase). As we mentioned earlier, the released glucose fuels the ATP-requiring fight-or-flight response to epinephrine.

Phosphorylation by activated protein kinase A alters the activities of many other proteins, including enzymes involved in glycolysis, a ribosomal protein, and a receptor for a neurotransmitter. Likewise, Ca^{2+} binds to many proteins in the cell, changing their activities. In addition to protein kinase C, Ca^{2+} -activated targets include proteins that bind to and organize actin microfilaments and microtubules, as well as troponin, a modulator of muscle contraction.

Different genes are transcribed

Cell surface receptors are involved in activating a broad range of gene expression responses. For example, the Ras signaling

pathway ends in the nucleus (see Figure 15.11). The final protein kinase enters the nucleus and phosphorylates a leucine zipper protein called AP-1. This activated protein stimulates the transcription of a number of genes involved in cell proliferation.

As we described earlier in this chapter, lipid-soluble hormones can diffuse directly through the plasma membrane and meet their receptors in the cytoplasm. Binding of the ligand allows the ligand/receptor complex to enter the nucleus, where it binds to hormone-responsive elements at the promoters of a number of genes. In some cases, transcription is stimulated, and in others it is inhibited.

In plants, light acts as a signal to initiate the formation of chloroplasts. Between this signal and response is a transcription-mediated signaling pathway. In bright sunlight, red wavelengths are absorbed by a receptor protein called phytochrome. We will say more about this important receptor

292 CHAPTER FIFTEEN

later in the book, but for now it is important to note that it is activated by red light. The activated phytochrome binds to cytoplasmic regulatory proteins, which enter the nucleus and bind to promoters for genes involved in the synthesis of important chloroplast proteins. Synthesis of these proteins is the key to the plant "greening".

Direct Intercellular Communication

Up to now, we have described how signals from the environment can influence a cell. But the environment of a cell in a multicellular organism is more than the extracellular medium. Most cells are in contact with their neighbors. In Chapter 5, we described how cells adhere to one another by the noncovalent interactions of recognition proteins protruding from the cell surface. There are also specialized cell junctions, such as tight junctions and desmosomes, that help "cement" the cells together (see Figure 5.7):

However, as we know from our own neighbors (and roommates), just being in proximity does not necessarily mean that there is functional communication. In this section, we look at the specialized junctions between cells that allow them to signal one another. In animals, these are gap junctions; in plants, they are plasmodesmata.

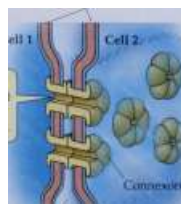
Animal cells communicate by gap junctions

Gap junctions are channels between adjacent cells that occur in many multicellular animals, occupying up to 25 percent of the area of the plasma membrane (Figure 15.18). Gap junctions traverse the 2-nm space between the plasma membranes of two cells (the "gap") by means of thin molecular channels called connexons. The walls of these tubes are composed of six subunits of an integral membrane protein appropriately named connexin. In two cells close to each other, two connexons come together, forming a channel that links the two cytoplasms. There may be hun-

Plasma membranes

Cell

The connexons of two cells come together to form a narrow (~1.5 nm gap junction, through which ions and small signal molecules can pass



"Gap" between cells (~2 nm)

75. 18 Gap Junctions Connect Animal Cells

An animal cell may contain hundreds of gap junctions connecting it to neighboring cells. Gap junctions are too small for proteins to pass through, but small molecules such as ATP, metabolic intermediates, amino acids, and coenzymes can be shared in this way.

Hundreds of these channels between a cell and its neighbors. The channels are quite narrow, about 1.5 nm in diameter. This is far too small for the passage of large molecules, such as proteins. But it is wide enough to allow small signal molecules and ions to pass between the cells. Experiments in which a labeled signal molecule or ion is injected into one cell show that it can readily pass into the adjacent cells if they are connected by gap junctions.

Gap junctions permit metabolic cooperation among linked cells. Such cooperation assures the sharing of important small molecules such as ATP, metabolic intermediates, amino acids, and coenzymes between cells. It may also assure that concentrations of ions and small molecules are similar in linked cells, thereby maintaining equivalent regulation of metabolism. It is not clear how important this is in many tissues, but it is known to be vital in some. For example, in the lens of the mammalian eye, only the cells at the periphery are close enough to the blood supply to allow diffusion of nutrients and wastes. But because lens cells are connected by large numbers of gap junctions, material can diffuse between them rapidly and efficiently.

There is evidence that signal molecules such as hormones and second messengers such as cAMP and IP₃ can move through gap junctions. If this is true, only a few cells would need to have receptors binding a signal in order for the stimulus to spread throughout the tissue. In this way, a tissue could have a coordinated response to the signal.

Plant cells communicate by plasmodesmata

Instead of gap junctions, plants have plasmodesmata, which are membrane-lined bridges spanning the thick cell walls that separate plant cells from one another. A typical plant cell has several thousand plasmodesmata.

Plasmodesmata differ from gap junctions in one fundamental way: Unlike gap junctions, in which the wall of the channel is made of integral proteins from the adjacent plasma membranes, plasmodesmata are lined by the fused plasma membranes themselves. Plant biologists are so familiar with the notion of a tissue as cells interconnected in this way that they refer to these continuous cytoplasms as a symplast (see Chapter 35).

The diameter of a plasmodesma is about 6 nm, far larger than the gap junction channel. But the actual space available for diffusion is about the same—1.5 nm—as with gap junctions. A look at the interior of the plasmodesma gives the reason for this reduction in pore size: A tubule called the desmotubule, apparently derived from the endoplasmic reticulum, fills up most of the opening of the plasmodesma (Figure 15.19). So, typically, only small metabolites and ions move between plant cells. This fact is important physiologically to plants, which lack the tiny circulatory vessels (capillaries) many animals use to bring gases and nutrients near enough to every cell.

Diffusion from cell to cell through plasma membranes is probably inadequate for hormonal responses in plants. Instead, they rely on more rapid diffusion through plasmodesmata.

Smooth

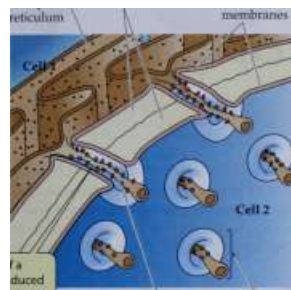
endoplasmic

reticulum

Cell walls

Fused plasma membranes

The channel size of a plasmodesma is reduced by the presence of the desmotubule and the proteins that adhere to it



Desmotubule

■ Plasmodesma

75.7 9 Plasmodesmata Connect Plant Cells

The desmotubule, derived from the endoplasmic reticulum, fills up most of the space inside plasmodesmata, leaving a tiny gap through which small metabolites and ions can pass.

Plasmodesmata ensure that all cells of a tissue respond to a signal at the same time. In C₄ plants (see Chapter 8), there are abundant plasmodesmata between the mesophyll and bundle sheath cells, helping to rapidly move the fixed carbon in the former cell type to the latter. A similar transport system, found at the junctions of nonvascular tissues and phloem, conducts organic solutes throughout the plant.

Plasmodesmata are not merely passive channels, but can be regulated. Plant viruses may infect cells at one location, then spread rapidly through a plant organ by plasmodesmata until they reach the plant's vascular tissue (circulatory system). These viruses, and even their RNA, would appear to be many times too large to pass through the plasmodesmal channel. But they get through, apparently by making "movement proteins" that increase the pore size temporarily while attached to the viral genome. Similar movement proteins are involved in transporting mRNAs between plant cells. This finding opens up the possibility of long-distance regulation of translation.



Chapter Summary

Signals

► Cells receive many signals from both the physical environment and other cells. Review Figures 15.1,15.2

► Signaling involves three steps: the binding of a signal by a receptor, the transduction of the signal within the cell, and the ultimate cellular response. Review Figure 15.3

Receptors

► Cells respond to signals only if they have specific receptor proteins that can bind to those signals. Review Figure 15.4

► Depending on the nature of the signal, the receptor may be at the plasma membrane or in the cytoplasm of the target cell. Review Figure 15.5

► Membrane receptors include ion channels, protein kinases, and G protein-linked receptors. Review Figures 15.6,15.7, 15.8

Transducers

► The events of signal transduction may be direct, occurring at the plasma membrane, or indirect, involving the formation of a second messenger. Review Figure 15.10

► Protein kinase cascades amplify a response to receptor binding. Review Figure 15.11

► Second messengers include cyclic AMP, the lipid-derived substances phosphatidylinositol and diacylglycerol, calcium ions, and the gas nitric oxide. Review Figures 15.12,15.13, 15.14,15.15

Effects

► The ultimate cell response to a signal may be the opening of membrane channels, the alteration of enzyme activities, or changes in gene transcription. Review Figures 15.16,15.17

Direct Intercellular Communication

► Animal cells can communicate directly, through small pores in their plasma membranes called gap junctions. Small molecules and ions can pass through these channels. Review Figure 15.18

► Plant cells are connected by somewhat larger pores called plasmodesmata, which traverse both membranes and cell walls. Review Figure 15.19

For Discussion

1. Like Ras itself, the various components of the Ras signaling pathway (see Figure 15.11) were discovered when tumors showed mutations in one or another of the components. What might be the biochemical consequences of mutations in the genes for (a) Raf and (b) MAP kinase that result in rapid cell division?

2. Cyclic AMP is a second messenger in many different responses, ranging from the sense of smell to the breakdown of glycogen. How can one messenger act in different ways in different cells?

3. Compare direct communication via plasmodesmata or gap junctions with ligand/receptor-mediated communication between cells. What are the advantages of one method over the other?

4. The tiny invertebrate Hydra has an apical region, which has tentacles, and a long, slender body. Hydra can reproduce asexually when cells on the body wall differentiate and form a bud, which breaks off as a new organism. Buds form only at a certain distance from the apex, leading to the idea that the apex releases a molecule that diffuses down the body and, at high concentrations (near the apex), inhibits bud formation. Hydra lacks a circulatory system, so the inhibitor must diffuse from cell to cell. If you had an antibody that binds to connexin and plugs up gap junctions, how would you show that the inhibitory factor passes through them?

16

Development:

Differential Gene Expression



IT IS A DAY IN THE NOT-TOO-DISTANT FUTURE. Decades of eating fatty foods, combined with a genetically based tendency to deposit cholesterol in his arteries, finally catch up with 60-year-old Don: A blood clot closes off the blood flow to part of his heart, leading to a heart attack and irreversible tissue damage. Today, Don would be faced with a long period of rehabilitation, taking medications to manage his weakened heart. Instead, his physicians inject undifferentiated embryonic stem cells directly into his heart. The cells differentiate into cardiac muscle cells, replacing the ones that were lost to oxygen starvation, and full heart function is restored.

Embryonic stem cells, which are cells from a very young mammalian embryo, are able to form an entire organism if separated from one another. These cells can be removed from an embryo and maintained indefinitely in the laboratory. If they could be genetically altered to make them acceptable for transplants, these cells could be a source of tissue replacement not only for damaged hearts, but for the pancreas in people with diabetes and the brain in people with Alzheimer's disease.

While the application of stem cells to medicine is not yet possible, considerable new knowledge about the molecular biology of development has emerged. Much of this knowledge has come from studies on organisms such as the fruit fly *Drosophila*, the roundworm *Caenorhabditis elegans*, frogs, sea urchins, and a flowering plant, *Arabidopsis thaliana*. As we saw in Chapter 14, the genomes of eukaryotes are surprisingly similar, and the cellular and molecular principles underlying their development also turn out to be similar. Thus discoveries from one organism aid us in understanding other organisms, including ourselves.

Two major conclusions have emerged from studies of development. The first is that all types of somatic cells—all cells except gametes—in an organism retain all of the genes that were present in the fertilized egg. In other words, cell differentiation does not result from a loss of DNA. The second is that cellular changes during develop-

An Early Embryo

This mammalian embryo has been opened up to show the undifferentiated stem cells.

ment and cell differentiation result from differential expression of genes. During development, the various mechanisms of transcriptional and translational control described in Chapter 14 and the signaling mechanisms described in Chapter 15 work together to produce a complex organism.

The Processes of Development

Development is a process in which an organism undergoes a series of progressive changes, taking on the successive forms that characterize its life cycle (Figure 16.1). In its earliest stages of development, a plant or animal is called an embryo. Sometimes the embryo is contained within a protective structure such as a seed coat, an eggshell, or a uterus. An embryo does not photosynthesize or feed actively; instead, it obtains its food from its mother directly or indirectly (by way of nutrients stored in the egg, for example). A series of embryonic stages may precede the birth of the new, independent organism. Most individual organisms continue to develop throughout their life cycle; development ceases only with death.



(a) Flowering plant

(*Arabidopsis*)

(b) Insect

(*Drosophila*)

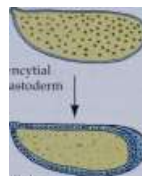


Nurse cells



Oocyte

Syncytial blastoderm



Cellular blastoderm



Larva



Pupa



Adult



^ 76.7 Stages of Development

Stages from embryo to adult are shown for a plant and an animal. Cell division and expansion, growth, cell differentiation, and the creation of the organs and tissues of the adult body are all part of the complex process of development.

Development consists of growth, differentiation, and morphogenesis

Growth (increase in size) occurs through cell division and cell expansion. It continues throughout the individual's life in some species, but reaches a more or less stable end point in others. Repeated mitotic divisions generate the multicellular body. In plants, unless the daughter cells become longer (expand) after they form, the embryo does not grow very much; thus in plant development, cell expansion begins shortly after the first divisions of the fertilized egg. In animal development, on the other hand, cell expansion is often slow to begin. The animal embryo may consist of

thousands of cells before it becomes larger than the fertilized egg.

Differentiation is the generation of cellular specializations; that is, differentiation defines the specific structure and function of a cell. Mitosis, as we have seen, produces daughter nuclei that are chromosomally and genetically identical to the nucleus that divides to produce them. However, the cells of an animal or plant body are obviously not all identical in structure or function. The human body, with its approximately 100 trillion (10^{14}) cells, consists of about 200 functionally distinct cell types—for example, muscle cells, blood cells, and nerve cells. This apparent contradiction results from regulation of the expression of various parts of the genome. When the embryo consists of only a few cells, each cell has the potential to develop in many different ways. As development proceeds, however, the possibilities available to individual cells gradually narrow, until each cell's fate is fully determined and the cell has differentiated.

Whereas differentiation gives rise to cells of different kinds, morphogenesis (literally, "creation of form") gives rise to the shape of the multicellular body and its organs. Morphogenesis results from pattern formation, the organization of differentiated tissues into specific structures. In plant development, cells are constrained by cell walls and do not move around, so organized division and expansion of cells are the major processes that build the plant body. In animals, cell movements are very important in morphogenesis. And in both plants and animals, programmed cell death is essential to orderly development.

In plants and animals alike, differentiation and morphogenesis result ultimately from the regulated activities of genes and their products, as well as the interplay of extracellular signals and their transduction in target cells.

As development proceeds, cells become more and more specialized

Marking specific cells of an early embryo with stains reveals which adult structures are derived from which part of the embryo. For instance, the shaded area of the frog embryo shown in Figure 16.2 normally becomes part of the skin of the tadpole larva. However, if we cut out a piece from this region and transplant it to another location on an early embryo, the type of tissue it becomes is determined by its new environment. The developmental potential of such cells—that is, their range of possible development—is thus greater than their actual fate, which is limited to what normally develops. Does embryonic tissue retain its broad developmental potential? Generally speaking, the answer is no. The developmental potential of cells becomes restricted fairly early in normal development. Tissue from a later-stage frog embryo, for example, if taken from a region fated to develop into brain, becomes brain tissue even if transplanted to parts of an early-stage embryo destined to become other structures. The tissue of the later-stage embryo is thus said to be determined: Its fate has been sealed, regardless of its surroundings. By contrast, the younger transplant tissue in Figure 16.2 has not yet become determined.

296 CHAPTER SIXTEEN

Determination precedes differentiation

Determination, the commitment of a cell to a particular fate, is a process influenced by the extracellular environment and the contents of the cell acting on the cell's genome. Determination is not something that is visible under the micro-scope—cells do not change appearance when they become determined. Determination is followed by differentiation, the actual changes in biochemistry, structure, and function that result in cells of different types. Differentiation often involves a change in appearance as well as function. Determination precedes differentiation. Determination is a commitment; the final realization of this commitment is differentiation.

The Role of Differential Gene Expression in Cell Differentiation

Differentiated cells are recognizably different from one another, sometimes visually as well as in their protein products. Certain cells in our hair follicles continuously produce keratin, the protein that makes up hair, nails, feathers, and porcupine quills. Other cell types in the body do not produce keratin. In the hair follicle cells, the gene that encodes keratin is transcribed; in most other cells in our body, that gene is not transcribed. Activation of the keratin gene is a key step in the differentiation of the hair follicle cells.

Generalizing from examples like this one, we may say that differentiation results from differential gene expression—that is, from the differential regulation of transcription, posttranscriptional events such as mRNA splicing, and translation (see Chapter 14).

Because the fertilized egg, or zygote, has the ability to give rise to every type of cell in the adult body, we say it is totipotent. Its genome contains instructions for all of the structures and functions that will arise throughout the life cycle. Later in the development of animals (and probably to a lesser extent in plants), the cellular descendants of the zygote lose their totipotency and become determined—that is, committed to form only certain parts of the embryo. Determined cells differentiate into specific types of specialized cells, such as nerve cells or muscle cells. When a cell becomes specialized, it is said to have differentiated.

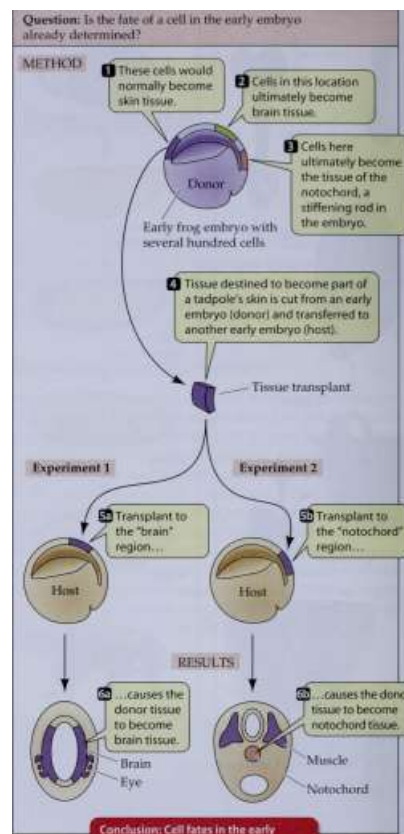
Differentiation usually does not include an irreversible change in the genome

Differentiation is irreversible in certain types of cells. Examples include the mammalian red blood cell, which loses its nucleus during development, and the tracheid, a water-conducting cell in vascular plants. Tracheid development culminates in the death of the cell, leaving only the pitted cell walls that formed while the cell was alive (see Chapter 34). In both of these extreme cases, the irreversibility of differentiation can be explained by the absence of a nucleus.

Generalizing about mature cells that retain functional nuclei is more difficult. We tend to think of plant differentiation as reversible and of animal differentiation as irreversible, but this is not a hard-and-fast rule. Why is differentiation apparently reversible in some cells but not in

EXPERIMENT

Question: Is the fate of a cell in the early embryo already determined?



Conclusion: Cell fates in the early embryo are not determined, but can change depending on the environment.

76.2 Developmental Potential in Early Frog Embryos

Cells that would be expected to form one kind of tissue can form completely different tissues when they are experimentally moved within the early embryo.

others? At some stage of development, do changes within the nucleus permanently commit a cell to specialization? For both higher plants and animals, the answer appears to be no. Under the right environmental circumstances, differentiation is reversible.

A food storage cell in a carrot root faces a dark future. It cannot photosynthesize or give rise to new carrot plants.

However, if we isolate that cell from the root, maintain it in a suitable nutrient medium, and provide it with appropriate chemical cues, we can "fool" the cell into acting as if it were a fertilized egg. It can divide and give rise to a normal carrot embryo and, eventually, a complete plant (Figure 16.3). Since the new plant is genetically identical to the somatic cell from which it came, we call the plant a clone.

The ability to clone an entire carrot plant from a differentiated root cell indicates that the cell contains the entire carrot genome and that it can express the appropriate genes in the right sequence. Many cells from other plant species show similar behavior in the laboratory, and this ability to generate a whole plant from a single cell has been invaluable in agricultural biotechnology (see Chapter 17).

These experiments with plants establish that a somatic cell is totipotent. A more direct demonstration that all the genetic material is present in differentiated cells has come from nuclear transplant experiments. Such experiments were first done on frogs by Robert Briggs and Thomas King, who asked whether the nuclei of early frog embryos had lost the ability to do what the totipotent zygote nucleus could do. They first removed the nucleus from an unfertilized egg, thus forming an enucleated egg. Then, with a very fine glass tube, they punctured a cell from an early embryo and drew up part of its contents, including the nucleus, which they injected into the enucleated egg.

More than 80 percent of these nuclear transplant operations resulted in the formation, from the egg and its new nucleus, of a normal early embryo. Of these embryos, more than half developed into normal tadpoles and, ultimately, normal adult frogs. These experiments showed that no information is lost from the nuclei of cells as they pass through the early stages of embryonic development, and that the cytoplasmic environment around a nucleus can modify its fate.

Similar experiments have been performed on rhesus monkeys, in which a single cell can be removed from an 8-celled

embryo and fused with an enucleated egg, allowing the nucleus of the embryonic cell to enter the egg cytoplasm. The resulting cell acts like a zygote, forming an embryo, which can be implanted into a foster mother, who ultimately gives birth to a normal monkey. Each of the remaining 7 cells from the original embryo can similarly give rise to offspring by the same cell fusion technique.

In humans, the totipotency of early embryonic cells permits both genetic screening and in vitro fertilization. An 8-cell human embryo can be isolated in the laboratory and a single cell removed to determine whether a harmful genetic condition is present (Chapter 18). Each remaining cell, being totipotent, can be stimulated to divide and form an embryo, which can be implanted into the mother, where it develops into an infant (Chapter 42).

Later frog nuclear transplant experiments by John Gur-don showed that a donor nucleus obtained from later stages in the frog's life could occasionally give rise to a normal tadpole, again showing totipotency. But successful cloning of animals was very difficult until the late 1990s,

EXPERIMENT

Question: Does a differentiated cell in an organ of a mature plant still retain totipotency—the ability to form all other tissues of the plant?

METHOD



Root of carrot plant

(°r

Ⓜ,

Ⓜ,

'©

o

Clumps of differentiated cells are removed from the root.

Differentiated cells are grown in a nutrient medium and dedifferentiate. Individual cells break off.

«-•-



Individual cells divide.

...and an embryo develops.

| The embryo is planted in a test tube medium.

RESULT



A new plant is produced.

Conclusion: Differentiated plant cells are totipotent.

76.3 Cloning a Plant

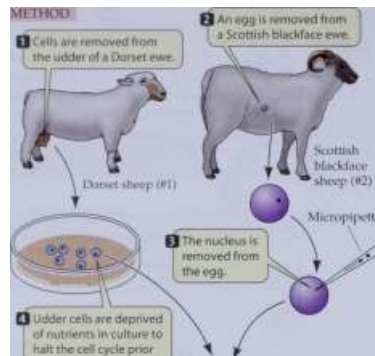
Differentiated, specialized food storage cells from the root of a carrot can be induced to dedifferentiate, act like embryonic cells, and form a new plant.

EXPERIMENT

Question: Are differentiated animal cells totipotent?

MI I HOD

I Cells are removed from the udder of a Dorset ewe



Q Udder cells are deprived of nutrients in culture to halt the cell cycle prior to DNA replication.



Q The udder cell and I enucleated egg are fused

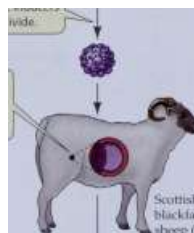
Y\

t

Stimulating mitotic inducers I⁺ causes the cell to divide.

o

Q An early embryo develops and is transplanted into a receptive ewe.



RESULTS

IpThe embryo develops and Dolly is born.

Scottish blackface sheep (#3)



_^

Dorset sheep, genetically identical to #1

Conclusion: Differentiated animal cells are totipotent in nuclear transplant experiments.

when Ian Wilmut and his colleagues at a biotechnology company in Scotland used the cell fusion procedure to clone sheep (Figure 16.4). Previous attempts to produce mammals by this method had worked, as in the rhesus monkey case, only if the donor nucleus was from an early



76.4 A Clone and Her Offspring

Although Dolly herself (right) is a clone with only one parent, she has mated and given birth to "normal" offspring (the lamb on the left), proving the genetic viability of cloned mammals.

embryo. Apparently, when mammalian donor cells were in the G₂ phase of the cell cycle and were fused with egg cytoplasm also in G₂, some extra DNA replication took place that created havoc with the cell cycle in the egg when it attempted to divide.

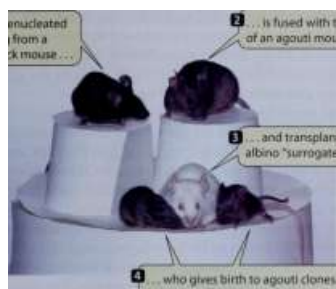
Wilmut took differentiated cells from a ewe's udder and starved them of nutrients for a week, thus halting the cells in G₁. After one of these cells was fused with an enucleated egg from a different breed of ewe, mitotic inducers in the egg cytoplasm (see Chapter 9) were stimulated and the donor nucleus entered S phase; the rest of the cell cycle proceeded normally. After several cell divisions, the resulting early embryo was transplanted into the womb of a surrogate mother. Out of 277 successful attempts to fuse adult cells with enucleated eggs, one lamb, named Dolly, survived to be born. DNA analyses confirmed that Dolly was genetically identical to the ewe from whose udder the donor nucleus had been obtained.

The purpose of Wilmut's experiment was to clone sheep that have been genetically programmed to produce products such as pharmaceuticals in their milk. The cloning procedure could make multiple, identical copies of sheep that are reliable producers of a drug such as α -1-antitrypsin, which is used to treat people with cystic fibrosis (see Figure 17.15).

The trick of starving donor cells for cloning has been applied to other mammals. Mice have been cloned using the cells lining the egg as a source of donor nuclei (Figure 16.5). Cows have been cloned to preserve a rare breed in New Zealand. Genetically engineered goats have been cloned to produce several useful proteins in their milk. This flurry of cloning has touched off a flurry of controversy, but cloning

An enucleated egg from a black mouse..

... is fused with the nucleus of an agouti mouse...



... and transplanted into an albino "surrogate mother" ..

. who gives birth to agouti clones.

76.5 Cloned Mice

Because so much is known about mouse genetics and molecular biology, cloned mice may be useful in studies of basic biology.

is not a new scientific concept. The idea of totipotency was accepted long before Dolly was born, but achieving it is an impressive technical achievement.

An example of nuclear totipotency gone awry occurs in a human tumor called a teratocarcinoma. Here, a differentiated cell dedifferentiates to form an unspecialized cell. Then it divides, forming a tumor, as occurs in most cancers. But some cells in the tumor redifferentiate to form specialized tissue arrangements. So the tumor can be a single mass of cells inside the abdomen, with some of the cells forming kidney tubes, others hair, and still others teeth! How this occurs is not clear.

Stem cells can be induced to differentiate by environmental signals

Totipotency implies that a differentiated cell stays that way because of its environment, and that appropriate environmental changes could result in a new pattern of differentiation. In normal development, a complex series of signals and their

transduction results in the patterns of differentiation we see in a newborn organism. If these signals could be described in enough detail, we should be able to understand how any cell type becomes any other.

Stem cells are undifferentiated, dividing cells that are found in adult animal tissues that need frequent cell replacement, such as skin, the inner lining of the intestine, and the blood system. As they divide, stem cells produce cells that differentiate to replace dead cells and maintain tissue homeostasis. In the body, stem cells have limited abilities to differentiate. The stem cells in bone marrow, for example, produce the various types of red and white blood cells, while stem cells in the nervous system produce the various differentiated nerve cells.

Can one kind of stem cell be manipulated by its environment to produce cells that differentiate into cells of another

tissue? The answer appears to be yes. For example, when stem cells of the brain were transplanted into the bone marrow of mice whose bone marrow stem cells had been depleted, they proceeded to act like bone marrow stem cells, producing blood cells. In the reverse experiment, bone marrow stem cells were implanted into the brains of mice, where they formed brain cells. These experiments indicate that the environment—presumably intercellular signals— determines what a stem cell will do.

The stem cell populations closest to totipotency are not the ones in adults, but those of the early embryo. In mice, these embryonic stem cells can be removed from an early embryo (called a blastocyst) and then induced to differentiate in some particular way. Normally, these cells are formed a few days after fertilization, and soon become determined as to what their fate will be in the developing embryo. Before then, however, they are virtually totipotent. Such cells can be grown indefinitely in the laboratory and, when injected back into a mouse blastocyst, will mix with the resident cells and differentiate to form all the cells of the mouse. This kind of experiment shows that they have not lost any of their developmental potential while growing in the laboratory.

Embryonic stem cells growing in the laboratory can be induced to differentiate if the right signal is provided (Figure 16.6). For example, treatment of mouse embryonic stem cells with a derivative of vitamin A causes them to form nerve cells, while other growth factors induce them to form blood cells, again demonstrating their developmental potential and the roles of environmental signals. This finding raises the possibility of using stem cell cultures as sources of differentiated cells for clinical medicine. A key advance toward this use has been the ability to grow human embryonic stem cells in the laboratory. The age of custom-made cells to replace ones lost to disease or injury is rapidly approaching.

Genes are differentially expressed in cell differentiation

Experiments such as nuclear transplants in frogs and sheep—as well as plant cell cloning—point to the conclusion of genome constancy or equivalence in all somatic cells of an organism. Molecular experiments have provided even more convincing evidence. For example, the gene for (3-glo-bin, one of the protein components of hemoglobin, is present and expressed in red blood cells as they form in the bone marrow of mammals. Is the same gene also present— but unexpressed—in nerve cells in the brain, which do not make hemoglobin?

Nucleic acid hybridization (see Figure 14.7) can provide an answer. A probe for the (3-globin gene can be applied to DNA from both brain cells and immature red blood cells (recall that mature red blood cells lose their nuclei and DNA). In both cases, the probe finds its complement, showing that the (3-globin gene is present in both types of cells.

On the other hand, if the probe is applied to cellular mRNA rather than cellular DNA, it finds (3-globin mRNA

300 CHAPTER SIXTEEN



Blastocyst

Inner cell mass

The early embryo, or blastocyst, is cultured in a nutrient medium.

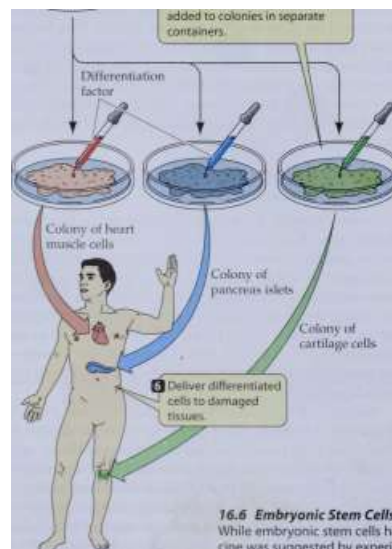
The outer layer collapses and the inner cell mass is freed from the embryo.

Chemicals are added to disaggregate the inner cell mass into smaller clumps.

Clumps of cells

Colonies of embryonic stem cells

J Each clump grows into a colony.



only in the red blood cells, and not in the brain cells. This result shows that the gene is expressed in only one of the two tissues. Many similar experiments have shown convincingly that differentiated cells lose none of the genes that were present in the fertilized egg.

What leads to this differential gene expression? One well-studied example is the conversion of undifferentiated muscle precursor cells, called myoblasts, into the large, multinucleated cells that make up mammalian skeletal muscle cells (called muscle fibers). The key event that starts this conversion is the expression of MyoDl (Myoblast Determining Gene 1). The protein product of this gene is a transcription factor (MyoDl) with a helix-loop-helix domain (see Chapter 14) that not only binds to promoters of the muscle-determining genes to stimulate their transcription, but also acts on its own promoter to keep its levels high in the myoblasts and in their descendants.

Strong evidence for the controlling role of MyoDl comes from experiments in which a gene containing an active promoter adjacent to MyoDl DNA is injected into the precursors of other cell types. For example, if MyoDl DNA is put into fat cell precursors, they are reprogrammed to become muscle cells. Genes such as MyoDl, which code for proteins that direct fundamental decisions in development, often by regulating genes on other chromosomes, usually code for transcription factors.

The Role of Polarity in Cell Determination

What initially stimulates the MyoDl promoter to begin transcription is not clear, but a chemical signal clearly is involved. In general, two overall mechanisms for producing such signals have been found. In cytoplasmic segregation, a factor within eggs, zygotes, or precursor cells is unequally distributed in the cytoplasm. After cell division, the factor ends up in some cells or regions of cells and not others. In induction, a factor is actively produced and secreted by certain cells to induce other cells to differentiate.

Polarity—the difference between one end of an embryo and the other—is obvious in development. Our heads are distinct from our feet, and the distal ends of our arms (wrists and fingers) differ from the proximal ends (shoulders). An animal's polarity develops early, even in the egg itself. Yolk may be distributed asymmetrically in the egg and embryo. In addition, other chemical substances may be confined to specific parts of the egg, or may be more concentrated at one pole than at the other.

In some animals, the original polar distribution of materials in the egg's cytoplasm changes as a result of fertilization. As cell division proceeds, the resulting cells contain unequal amounts of the materials that were not distributed uniformly in the zygote. As we learned from the work on

76.6 Embryonic Stem Cells, Differentiation, and Medicine

While embryonic stem cells have been cultured from humans, their potential use in medicine was suggested by experiments in mice. This technique is under intensive investigation.

o Special differentiation factors are added to colonies in separate containers.

Colony of cartilage cells

EXPERIMENT

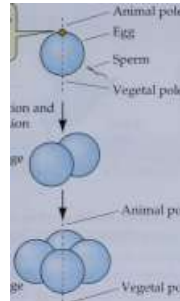
Question: Do different regions in the fertilized egg and the embryo have different developmental potential?

The position of the polar body which later disintegrates, establishes the animal pole.

Fertilization and cell division

2-Cell stage

4-Cell stage



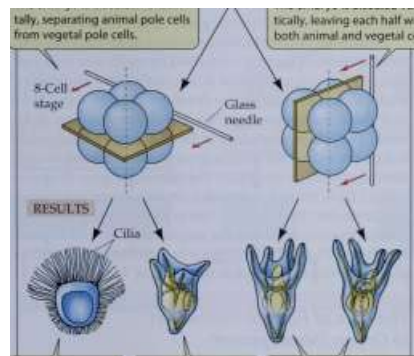
Animal pole

Vegetal pole

METHOD

The embryo is bisected horizontally, separating animal pole cells from vegetal pole cells.

The embryo is bisected vertically, leaving each half with both animal and vegetal cells.



Cells from only the animal pole remain embryonic.

Cells from only the vegetal pole make a small, abnormal larva.

Cells from both the animal and vegetal poles make small but normal larvae.

Conclusion: The animal and vegetal pole halves differ in their developmental potential.

7 6.7 Early Asymmetry in the Embryo

The upper (animal pole) and lower (vegetal pole) halves of very early sea urchin embryos differ in their developmental potential. Cells from both halves are necessary to produce a normal larva.

cloning, cell nuclei do not always undergo irreversible changes during early development; thus we can explain some embryological events on the basis of the cytoplasmic differences in cells.

Even a structure as apparently uniform as a sea urchin egg has polarity. A striking difference between cells can be

DEVELOPMENT: DIFFERENTIAL GENE EXPRESSION 301

demonstrated very early in embryonic sea urchin development. The development of embryos that have been divided in half at the 8-cell stage depends on how they are separated. If the embryo is split into "left" and "right" halves, with each half containing cells from both the upper and the lower halves, normal-shaped but dwarfed larvae develop. If, however, the cut separates the upper four cells from the lower four, the result is different. The upper four cells develop into an abnormal early embryo with large cilia at one end that cannot form a larva. The lower four cells develop into small, somewhat misshapen larvae with an oversized gut (Figure 16.7). These results show that for fully normal development, factors from both the upper and lower parts of the embryo are necessary.

This and many other experiments established that certain materials, called cytoplasmic determinants, are distributed unequally in the egg cytoplasm, and that these materials play a role in directing the embryonic development of many organisms.

The Role of Embryonic Induction in Cell Determination

Experimental work on developing embryos has clearly established that in many cases, the fates of particular tissues are determined by interactions with other specific tissues in the embryo. In developing animal embryos there are many such instances of induction, in which one tissue causes an adjacent tissue to develop in a particular manner. These effects are mediated by intercellular biochemical communication—that is, signal transduction mechanisms. We will describe two examples of such induction: one in the developing vertebrate eye, and the other in a developing reproductive structure in the nematode *C. elegans*.

Tissues direct the development of their neighbors by secreting inducers

The development of the lens in the vertebrate eye is a classic example of induction. In a frog embryo, the developing forebrain bulges out at both sides to form the optic vesicles, which expand until they come into contact with the cells at the surface of the head (Figure 16.8). The surface tissue in the region of contact with the optic vesicles thickens, forming a lens placode. The lens placode bends inward, folds over on itself, and ultimately detaches from the surface to produce a structure that will develop into the lens.

If the growing optic vesicle is cut away before it contacts the surface cells, no lens forms. Placing an impermeable barrier between the optic vesicle and the surface cells also prevents the lens from forming. These observations suggest that the surface tissue begins to develop into a lens when it receives a signal—an embryonic inducer—from the optic vesicle.

The interaction of tissues in eye development is a two-way street: There is a "dialogue" between the developing optic vesicle and the surface tissue. The optic vesicle induces lens development, and the developing lens determines the

The optic vesicle bulges out from the forebrain.



302 CHAPTER SIXTEEN

76.8 Inducers in the Vertebrate Eye

The eye of a frog develops as inducers take their turns.

size of the optic cup that forms from the optic vesicle. If head surface tissue from a frog species with small eyes is grafted over the optic vesicle of one with large eyes, both lens and optic cup are of intermediate size.

The developing lens also induces the surface tissue over it to develop into a cornea, a specialized layer that allows light to pass through and enter the eye. Thus a chain of inductive interactions participates in the development of the parts required to make an eye. Induction triggers a sequence of gene expression in the responding cells. Tissues do not induce themselves; rather, different tissues interact and molecularly induce each other. We will return to embryonic induction in Chapter 43.

Single cells can induce changes in their neighbors

The tiny nematode roundworm *Caenorhabditis elegans* is used as a model organism in many biological studies, but it is especially useful for studying development. It normally lives in the soil, where it feeds on bacteria, but can also grow in the laboratory if supplied with its food source. The entire process of development from egg to larva takes about 8 hours. It is easily observed using a low-magnification dissecting microscope because the body covering is transparent (Figure 16.9a). Because *C. elegans* is easy to culture, develops rapidly, and is easily observed, it is a favorite experimental organism. The development of *C. elegans* does not vary, so it has been possible to identify the source of each of the 959 somatic cells of the adult form.

The adult nematode is hermaphroditic, containing both male and female reproductive organs. It lays eggs through a pore called the vulva on the ventral surface. During development, a single cell, called the anchor cell, induces the vulva to form. If the anchor cell is destroyed by laser surgery, no vulva forms. The eggs develop inside the parent, and a "bag of worms" that eventually consume the parent results.

The anchor cell controls the fates of six cells on the animal's ventral surface through two molecular switches. Each of these cells has three possible fates: It may become a primary vulval precursor, a secondary vulval precursor, or simply part of the worm's surface—an epidermal cell (Figure 16.9b).

The anchor cell produces an inducer that diffuses out of the cell and interacts with adjacent cells. Cells that receive enough of the inducer become vulval precursors; cells slightly farther from the anchor cell become epidermis. The first molecular switch, controlled by the inducer from the anchor cell, determines whether a cell takes the "track" toward becoming part of the vulva or the track toward becoming epidermis.

The optic vesicle induces overlying tissue to form the lens placode.

The optic cup forms and induces lens formation.

The developing lens separates from surface tissue and induces it to form the cornea.

Developing forebrain



Cornea

Surface tissue Lens placode tissue

Lens forming

Lens

The cell closest to the anchor cell, having received the most inducer, differentiates into the primary vulval precursor and apparently produces its own inducer, which acts on the two neighboring cells and directs them to become secondary vulval precursors. Thus the primary vulval precursor cell controls a second molecular switch, determining whether a vulval precursor will take the primary track or the secondary track. The two inducers control the activation or inactivation of specific genes in the responding cells.

There is an important lesson to draw from this example: Much of development is controlled by molecular switches that allow a cell to proceed down one of two alternative tracks. One challenge for the developmental biologist is to find these molecular switches and determine how they work. The primary inducer for the *C. elegans* vulva appears to be a growth factor homologous to the mammalian epidermal growth factor (EGF). The nematode growth factor, called LIN-3, binds to a receptor on the surface of a vulval precursor cell. This binding sets in motion a signal transduction cascade involving the ras protein and MAP kinases (see Figure 15.11). The end result is increased transcription of genes involved in the differentiation of vulval cells.

The Role of Pattern Formation in Organ Development

Pattern formation, the spatial organization of a tissue or organism, is inextricably linked to morphogenesis, the appearance of body form. The differentiation of cells is beginning to be understood in terms of molecular events, but how do molecular events contribute to the organization of multitudes of cells into specific body parts, such as a leaf, a flower, a shoulder blade, or a tear duct?

Some cells are programmed to die

Apoptosis is programmed cell death, a series of events caused by the expression of certain genes (see Figure 9.20). Some of these "death genes" have been pinpointed, and related ones have been found in organisms as diverse as nematodes and humans.

Apoptosis is vital to the normal development of all animals. For example, the nematode *C. elegans* produces precisely 1,090 somatic cells as it develops from a fertilized egg to an adult (see Figure 16.9). But 131 of these cells die. The sequential expression of two genes called *ced-4* and *ced-3*

(a) The nematode *C. elegans*

DEVELOPMENT: DIFFERENTIAL GENE EXPRESSION 303

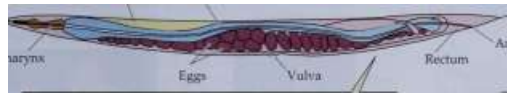
-1 mm



Internal structures are simple and clearly visible.

Ovary

Intestine



Anus

76.9 Induction during Vulval Development in *C. elegans*

(a) In the nematode *Caenorhabditis elegans*, it has been possible to trace all divisions of a single cell to the 959 cells found in the fully developed adult. (6) In vulval development, two secreted proteins act as the primary and secondary inducers. The gene activation patterns triggered by these switches determine cell fate.

Pharynx

The hermaphrodite has both male and female reproductive structures and reaches its adult stage just 3.5 days after fertilization of the egg.

(b)



Primary inducer activates gene 1



The primary inducer, produced by the anchor cell, activates a gene whose products determine that cells will develop as vulval precursors rather than epidermal cells.

The secondary inducer activates another gene, thus determining that cells will develop as secondary precursors.



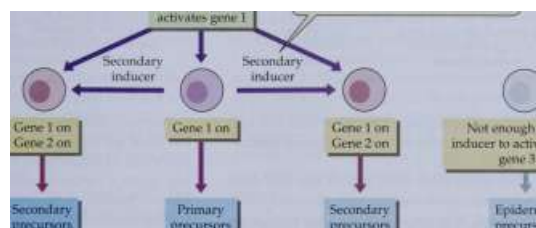
Not enough primary

inducer to activate gene 1:

gene 3 on

Epidermal precursors

Epidermal precursors



Not enough primary

inducer to activate gene 1:

gene 3 on

Secondary precursors

Epidermal precursors

r

n n *tt-i stt* rtt* rn

Epidermis

Vulva

Epidermis

(for cell death) appears to control this process. In the nervous system, for example, there are 302 nerve cells that come from 405 precursors; thus 103 cells undergo apoptosis. If the protein coded for by either *ced-3* or *ced-4* is nonfunctional, all 405 cells form neurons, and disorganization results. A third gene, *ced-3*, codes for an inhibitor of apoptosis: that is, its protein blocks the function of the *ced-4* gene. So, where cell death is required, *ced-3* and *ced-4* are active and *ced-3* is inactive; where cell death does not occur, the reverse is true.

Remarkably, a similar system of cell death genes acts in humans. During early development, human hands and feet look like tiny paddles—the fingers and toes are linked by webbing. Between days 41 and 56 of development, the cells in the webbing die, freeing the individual fingers and toes (Figure 16.10). The protein—an enzyme called caspase—that stimulates this apoptosis is similar in amino acid sequence to the protein encoded by *ced-3*, and a human protein (*bcl-2*) that inhibits apoptosis is similar to *ced-9*. So humans and



41 days after fertilization: Genes

for programmed cell death are expressed in the tissue between the digits.



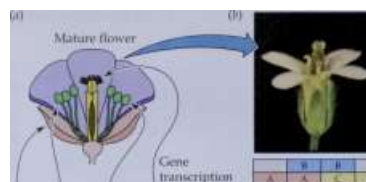
56 days after fertilization:

Apoptosis is complete. Cells of the digits have absorbed the remains of the dead cells.

16.10 Apoptosis Removes the Webbing between Fingers

Early in the second month of human development, the webbing connecting the fingers is removed by apoptosis, freeing the individual fingers.

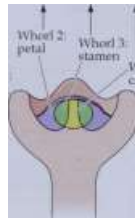
304 CHAPTER SIXTEEN



Whorl 1: sepal \

76.77 Organ Identity Genes in Arabidopsis Flowers

(a) The four organs of a flower (carpel, stamen, petals, and sepals) grow in whorls that develop from meristems. (b) When a homeotic mutation occurs, one type of organ replaces another. Such mutations helped scientists decipher the pattern of gene expression that gives rise to normal flowers.



Whorl 4: carpel

Early flower

differentiation

(meristems)

Wild-type Arabidopsis flower



Mutation of class B: No petals or stamens; sepals and carpels instead



Mutation of class A: No petals or sepals; stamens and carpels instead



Mutation of class C: No stamens or carpels; petals and sepals instead

nematodes, two creatures separated by more than 600 million years of evolutionary time, have similar genes controlling programmed cell death.

Apoptosis plays many other roles in your life. The dead cells that form the outermost layer of your skin and those from the uterine wall that are lost during menstruation have undergone apoptosis. White blood cells live only a few months in the circulation, then undergo apoptosis (see Figure 9.20). In a form of cancer called follicular large-cell lymphoma, these white blood cells do not die, but continue to divide. The reason is a mutation that causes the overexpression of *bcl-2*, the gene that inhibits cell death.

Plants have organ identity genes

Like animals, plants have organs—for example, leaves and roots. Many plants form flowers, and many flowers are composed of four types of organs: sepals, petals, stamens, and carpels. These floral organs occur in whorls, which are groups of each organ around a central axis. The whorls develop from groups of cells called meristems in the shape of domes, which develop at growing points on the stem (Figure 16.11rt). How is the identity of a particular whorl determined? The answer appears to lie in the activities of a group of genes.

These genes have been best described in *Arabidopsis thaliana*, also called mouse-ear cress. This plant is very useful for studies of development because of its small size, abundant seed production (over 1,000 per plant), rapid development (from seed to plant to seed in 6 weeks), and small genome (10 chromosomes with 80 million base pairs of DNA). Finally, it is easy to produce mutations in this plant by treating the seeds with mutagens.

Normal *Arabidopsis* flowers have four whorls of organs, but there are mutant strains that have the wrong organs in particular whorls (Figure 16.11b). Such mutations, in which one organ is replaced with another, are called homeotic mutations. Studies on three mutant strains led to a model for the determination of floral organ identity. This model involves three organ identity genes, each of which is expressed in two of the whorls:

- ▶ A class A gene is expressed in whorls 1 and 2 (which normally form sepals and petals, respectively).
- ▶ A class B gene is expressed in whorls 2 and 3 (which normally form petals and stamens, respectively).

- A class C gene is expressed in whorls 3 and 4 (which normally form stamens and carpels, respectively).

Nucleic acid hybridization confirmed these locations for the mRNA transcripts of the three genes.

Not surprisingly, these three genes code for transcription factors, which are active as dimers. Possibly, gene regulation in these cases is combinatorial—that is, the composition of the dimer determines which other genes will be activated by the transcription factor. For example, a dimer made up only of transcription factor A would activate transcription of the genes that make sepals; a dimer made up of A and B would result in petals, and so forth.

A gene called *leafy* appears to control the transcription of the ABC genes. Plants with the *leafy* mutation are just that—they don't make flowers. The protein product of this gene acts as a transcription factor stimulating genes A, B, and C so that they produce the flower (Figure 16.12).

In addition to being fascinating to biologists, organ identity genes have caught the eye of horticultural and agricul-

tural scientists. Flowers filled with petals instead of stamens and carpels often have mutations of the C genes. Many of the foods that make up the human diet come from fruits and seeds, such as the grains of wheat, rice, and corn. These fruits and seeds form from the carpels (the female reproductive organs) of the flower. Genetically modifying the number of these organs on a particular plant could increase the amount of grain a crop could produce.

Plants and animals use positional information

Certain cells in both plants and animals appear to "know" where they are with respect to the body as a whole. This spatial "sense" is called positional information. In plants, the pattern of development of two major types of conducting tissue suggested to scientists long ago that distance from the body surface may play a role in their formation. Cells destined to become water conductors are farther from the body surface than are those destined to become conductors of sucrose, a product of photosynthesis. Thus those cells destined to become water conductors are exposed to lower concentrations of O_2 and higher concentrations of CO_2 . These differences may help determine which genes are expressed in which parts of the stem and root.

Recently it has been suggested that cells on the surface of the stem secrete a protein or other signal that is more concentrated close to the surface than deeper in the stem. Other signals may diffuse from the stem tip and root tip, establishing positional information along the plant's axis. These signals are called morphogens.

The wing of a chick develops from a round bud. The cells that become the bones and muscles of the wing must receive positional information. If they do not, the limbs will be totally disorganized (imagine fingers growing out of your shoulders). Three groups of cells—one at the junction of the bud and the body, a second at the tip of the bud, and a third on the surface of the bud—produce different morphogens that diffuse through the bud. Each cell in the bud receives unique concentrations of each morphogen. The



Wild-type

Leafy mutant

16.12 A Nonflowering Mutant

Mutations in the *leafy* gene of *Arabidopsis* prevent the transcription of the ABC genes, and the resulting plant does not produce any flower.

first morphogen determines the proximal-distal ("shoulder to fingertip") axis of the wing, the second determines the anterior-posterior ("thumb to little finger") axis, and the third determines the dorsal-ventral ("palm to knuckles") axis.

The signaling pathways involved in limb development have been conserved through animal evolution. Comparative genomic studies have revealed developmental signaling pathways using homologous morphogen proteins in organisms ranging from nematodes to humans.

The Role of Differential Gene Expression in Establishing Body Segmentation

Another experimental subject that developmental biologists have used to study pattern formation is the fruit fly, *Drosophila*. Insects (and many other animals) develop a highly modular body composed of different types of segments. Complex interactions of different sets of genes underlie the pattern formation of segmented bodies.

Unlike the body segments of segmented worms such as earthworms, the segments of the *Drosophila* body are clearly different from one another. The adult fly has a head (composed of several fused segments), 3 different thoracic segments, 8 abdominal segments, and a terminal segment at the posterior end. The 13 seemingly identical segments of the *Drosophila* larva correspond to these specialized adult segments. Several types of genes are expressed sequentially in the embryo to

define these segments. The first step in this process is to establish the polarity of the embryo.

Maternal effect genes determine polarity

In *Drosophila* eggs and larvae, polarity is based on the distribution of morphogens, of which some are mRNA's and some are proteins. These molecules are products of specific maternal effect genes in the mother and are distributed to the eggs, often in a nonuniform manner. The maternal effect genes are transcribed in the nurse cells, which surround and nurture the developing egg and are localized at certain specific regions of the egg as it forms. Maternal effect genes produce their effects on the embryo regardless of the genotype of the father. Their products determine the dorsal-ventral (back-belly) and anterior-posterior (head-tail) axes of the embryo.

The fact that these morphogens specify these axes has been established by the results of experiments in which cytoplasm was transferred from one egg to another. Females homozygous for a particular mutation of the maternal effect gene *bicoid* produce larvae with no head and/or no thorax. However, if eggs of homozygous *bicoid* mutant females are inoculated at the anterior end with cytoplasm from the anterior region of a wild-type egg, the treated eggs develop into normal larvae, with heads developing from the part of the egg that receives the wild-type cytoplasm. Conversely, removal of 5 percent or more of the cytoplasm from the anterior of a wild-type egg results in an abnormal larva that looks like a *bicoid* mutant larva.

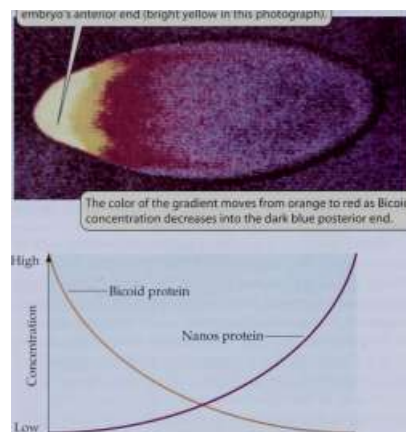
306 CHAPTER SIXTEEN

Thorax Abdomen



Head

The concentration of Bicoid protein is highest at the embryo's anterior end (bright yellow in this photograph).



Anterior of embryo

Posterior of embryo

16.13 Bicoid and Nanos Protein Gradients Provide Positional Information

The anterior-posterior axis of *Drosophila* arises from morphogens produced by the maternal effect genes *bicoid* and *nanos*. The mRNA's are translated at the ends of the larva, and the resulting gradient controls the developing body's polarity.

Another maternal effect gene, *nanos*, plays a comparable role in the development of the posterior end of the larva. Eggs from homozygous *nanos* mutant females develop into larvae with missing abdominal segments. Injecting a *nanos* egg with cytoplasm from the posterior region of a wild-type egg allows normal development. These findings show that, in wild-type larvae, the overall framework of the anterior-posterior axis is laid down by the activity of these two maternal effect genes (Figure 16.13).

After the axes of the embryo are determined, the next step in pattern formation is to determine the larval segments.

Segmentation and homeotic genes act after the maternal effect genes

The number, boundaries, and polarity of the larval segments are determined by proteins encoded by the segmentation genes. These genes are expressed when there are

about 6,000 nuclei in the embryo. The nuclei all look the same, but in terms of gene expression, they are not.

The maternal effect genes set the segmentation genes in motion. Three classes of segmentation genes act, one after the other, to regulate finer and finer details of the segmentation pattern (Figure 16.14):

► First, gap genes organize large areas along the anterior-posterior axis. Mutations in gap genes result in gaps in the body plan—the omission of several larval segments.

► Second, pair rule genes divide the embryo into units of two segments each. Mutations in pair rule genes result in embryos missing every other segment.

► Third, segment polarity genes determine the boundaries and anterior-posterior organization of the segments. Mutations in segment polarity genes result in segments in which posterior structures are replaced by reversed (mirror-image) anterior structures.

► Finally, after the basic pattern of segmentation has been established by the segmentation genes, differences between the segments are mediated by the activities of homeotic genes. These genes are expressed in different combinations along the length of the body and tell each segment what to become. Homeotic genes are analogous to the organ identity genes of plants.

The maternal effect, segmentation, and homeotic genes interact to "build" a *Drosophila* larva step by step, beginning with the unfertilized egg.

I Maternal effect genes

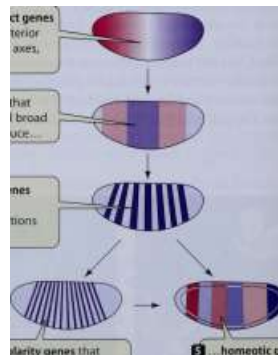
determine anterior and posterior axes, and induce...

Q ...gap genes that

define several broad areas and induce...

Q ...pair rule genes

that predict segment locations and induce...



I... segment polarity genes that begin segmentation and define subregions within segments and...

..homeotic genes

that define the role of each segment.

16.14 A Gene Cascade Controls Pattern Formation in the *Drosophila* Embryo

Gap, pair rule, and segment polarity genes are collectively referred to as the segmentation genes. The shading shows the locations of their gene products in the embryo.



Drosophila development results from a transcriptionally controlled cascade

One of the most striking and important observations about development in *Drosophila* —and in other animals—is that it results from a sequence of changes, with each change triggering the next. The sequence, or cascade, is largely controlled at the levels of transcription and translation.

In general, unfertilized eggs are storehouses of mRNA's, which are made prior to fertilization to support protein synthesis during the early stages of embryo development. Indeed, early embryos do not carry out transcription. After several cell divisions, mRNA production begins, forming the mRNA's needed for later development.

Some of the prefabricated mRNA's in the egg provide positional information. Before the egg is fertilized, mRNA for the Bicoid protein is localized at the end destined to become the anterior end of the fly. After the egg is fertilized and laid, nuclear divisions begin. (In *Drosophila*, cell divisions do not begin right away; until the thirteenth cell division, the embryo is a single, multinucleated cell called a syncytium.) At this early point, bicoid mRNA is translated, forming Bicoid protein, which diffuses away from the anterior end, establishing a gradient. At the posterior end, Nanos forms a gradient in the other direction. Thus each nucleus in the developing embryo is exposed to a different concentration ratio of Bicoid and Nanos proteins.

The two morphogens regulate the expression of the gap genes, although in different ways. Bicoid protein affects transcription, while Nanos affects translation. The high concentrations of Bicoid protein in the anterior portion of the egg turn on a gap gene called hunchback, while simultaneously turning off another gap gene, Krüppel. Nanos at the posterior end reduces the translation of hunchback, so a difference in concentration of these two gap gene products at the two ends is established.

The proteins encoded by the gap genes control the expression of the pair rule genes. Many pair rule genes in turn encode transcription factors that control the expression of the segment polarity genes, giving rise to a complex, striped pattern (see Figure 16.14) that foreshadows the segmented body plan of *Drosophila*.

By this point, each nucleus of the embryo is exposed to a distinct set of transcription factors. The segmented body pattern of the larva is established even before any sign of segmentation is visible. When the segments do appear, they are not all identical, because the homeotic genes specify the different structural and functional properties of each segment. Each homeotic gene is expressed over a characteristic portion of the embryo.

Let's turn now to the homeotic genes and see how their mutation can alter the course of development.

Homeotic mutations produce large-scale effects

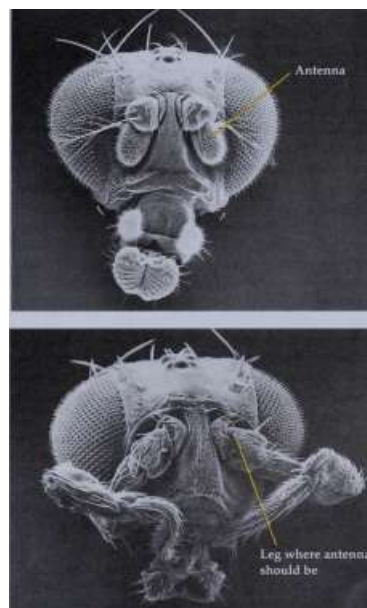
Two bizarre homeotic mutations in *Drosophila* are the Antennapedia mutation, in which legs grow in place of antennae (Figure 16.15), and the bithorax mutation, in which an extra pair of wings grows in a thoracic segment (see Figure 24.9).

Edward Lewis at the California Institute of Technology found that bithorax was not a single gene, but a cluster of genes, each one determining the functional identity of a segment. Moreover, the genes were lined up along the chromosome in the order of the segments they determined: Genes at the beginning of the cluster determined thoracic segments, then the next genes determined the upper abdomen, and so on. A similar cluster of genes—the ones that are mutated in the Antennapedia flies—was found to determine the identities of the segments at the front of the fly. Lewis predicted that all of these genes might have come from the duplication of a single gene in an ancestral, unsegmented organism.

Molecular biologists confirmed Lewis's prediction. Using a nucleic acid hybridization probe for part of one of the genes of the cluster, several scientists found the probe binding not only to its own gene, but also to adjacent genes in its cluster and to genes in the other homeotic cluster. In

(a)

(b)

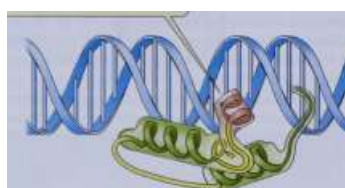


16.15 A Homeotic Mutation in *Drosophila*

Mutations of the homeotic genes cause body parts to form on inappropriate segments, (a) A wild-type fruit fly. (b) An Antennapedia mutant fruit fly.

308 CHAPTER SIXTEEN

This helix recognizes a sequence in the wide groove of DNA.



76.76 A Homeodomain Binds to DNA

The homeodomain region has a helix-turn-helix motif. There are three α -helices, one of which is involved in recognition of a DNA

sequence.

other words, there are DNA sequences that are common to all the homeotic genes in both clusters.

Homeobox-containing genes encode transcription factors

The 180-base-pair DNA sequence that is common to the *bitlwrax* and *Antennapedia* gene clusters is called the homeo-box. It encodes a 60-amino-acid sequence, called the homeodomain, which binds to DNA (Figure 16.16). This sequence turns out to be present in other proteins involved in *Drosophila* pattern formation, such as *bicoid*. In all cases, the DNA-binding region of the protein has a helix-turn-helix motif (see Chapter 14). Each type of homeodomain recognizes a specific DNA sequence in target genes. The *bicoid* homeodomain, for example, recognizes TCCTAATCCC. What do these proteins do when they recognize their target sequence in DNA? Not surprisingly, they are transcription factors, affecting genes involved in development. For example, *Bicoid* protein binds to promoters for the gap gene *hunchback*, activating its transcription. *Hunchback* protein is also a transcription factor, binding to enhancers and activating genes involved in head and thorax formation. In this way, the homeodomain proteins produce the cascade of events that controls *Drosophila* development.

fly development, those probes were applied to other organisms. Soon, homeoboxes were found in over a hundred proteins from organisms as diverse as nematodes, frogs, mice, chickens, plants, and humans. As developmental biologists have described more and more intricate developmental systems at the molecular level, they have found that these systems are present in other organisms as well. These systems are an example of gene conservation. Comparative genomic studies have shown that, like the genes involved in biochemical pathways, the genes controlling development have much in common among different organisms. There may be only small differences that have turned one species into another, or a fin into a limb. This comparative approach has spawned a new discipline called evolutionary developmental biology, or "evo-devo."

homeotic gene clusters. As Lewis predicted, homeobox genes are involved in many developmental pathways. In the mouse, for example, there are 38 such genes in four clusters, each located on a different chromosome. As do the homeobox genes in *Drosophila*, these Hox genes control the development of specific regions of the mouse embryo, and are arranged in the same order on the chromosome as they are expressed, from anterior to posterior, in the developing animal (Figure 16.17). These four clusters are present in all vertebrate animals, and apparently arose from repeated duplications, followed by small mutations, of a single ancestral gene. This gene was recently found in the lancelet, a tiny marine organism thought to be the simplest living member of the chordates, a lineage that includes the vertebrates. As we will see in Chapter 24, gene duplication is a common mechanism of evolutionary change. Here, it allowed the formation of gene clusters that could determine increasingly complex organisms.

Drosophila

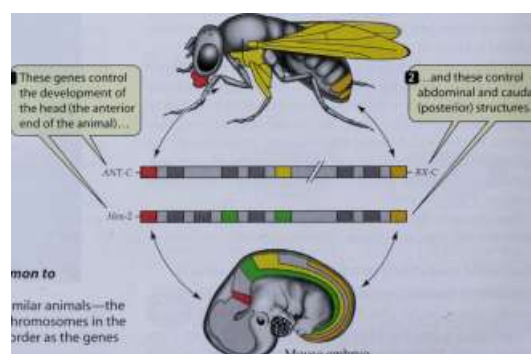
These genes control the development of the head (the anterior end of the animal)...

Evolution and Development

Once DNA probes had identified the homeobox sequences involved in fruit

76.77 Homeobox-Containing Genes Are Common to Vastly Different Organisms

In both the fruit fly and the mouse—two very dissimilar animals—the homeobox-containing genes are lined up on the chromosomes in the same order, and produce their effects in the same order as the genes along the anterior-posterior axis.



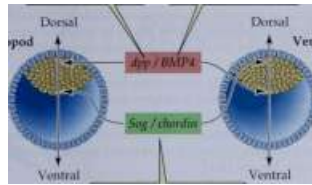
Mouse embryo

Q In arthropods, this gene determines what will be dorsal...

5 ••but its homolog in vertebrates determines what will be ventral.

Dorsal

Arthropod



Vertebrate

Ventral

Ventral

Dorsal

circulatory Y system

f

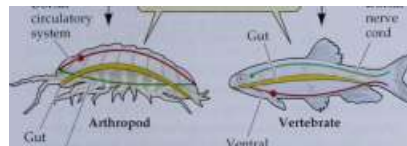
These two homologous genes do just the opposite.

o Expression of these four genes results in a shift in adult body plans.

Dorsal

nerve

cord



Vertebrate

Gut

Ventral nerve cord

Ventral

circulatory

system

CONSERVATION OF A GENE FOR EYE FORMATION. The eye of

an insect is very different in form and function from the eye of a mammal. Yet similar genes are involved in their formation. Mutations of a *Drosophila* gene called *eyeless* result in the reduction or absence of eyes in the adult fly. The protein encoded by the wild-type gene is a transcription factor that binds to promoters in over 1,500 different genes involved in eye formation. The homolog of *eyeless* in vertebrates, *Pax6*, is also involved in eye formation. In fact, mutations in the human version of *Pax6* result in a disease called *aniridia*— the partial or total absence of the iris of the eye. Remarkably, the *Pax6* and *eyeless* genes can be swapped for one another and yet remain functional in the other organism's development. This conservation of a global role for a gene for eye formation suggests that these "general instructions" evolved early and have been used repeatedly as different types of eyes have evolved.

dorsal and ventral. In 1822, Geoffrey Saint-Hillaire noticed that in arthropods, such as the lobster, the nerve cord is on the same side as the mouth (ventral), with the circulatory system on the opposite side (dorsal) and the digestive system between the two. This pattern is the inverse of the one in vertebrates, in which the circulatory system is ventral and the nerve cord is dorsal (Figure 16.18). Molecular biologists have now described this "flip" in terms of genes that act during development. The signals for dorsal-ventral determination in arthropods and in vertebrates are the same; it's just that they have opposite mean-

16.18 Shifts of Homologous Gene Expression and Shift in Body Plans of Arthropods and Vertebrates

Although arthropods and vertebrates have similar and analogous genes governing development, these genes determine opposite locations for the nervous and circulatory systems.

ings! So the protein chordin determines dorsal identity in vertebrates, but its homolog, *Sog*, specifies the ventral region of arthropods. Likewise, *BMP4* is a ventral determiner in vertebrates, but its relative, *Dpp*, is a dorsal determiner in arthropods. These findings suggest that the difference in body plan between arthropods and vertebrates involved an inversion of the axis of expression of these genes during evolution.

Chapter Summary

The Processes of Development

- ▶ A multicellular organism develops through a series of embryonic stages and eventually into an adult. Development continues until death. Review Figure 16.1
- ▶ Growth results from a combination of cell division and cell expansion.
- ▶ Differentiation produces specialized cell types.
- ▶ Morphogenesis—the creation of the overall form of the multicellular organism—is the result of pattern formation.
- ▶ In many organisms, the fates of the earliest embryonic cells have not yet been decided. These early embryonic cells may develop into different tissues if transplanted to other parts of an embryo. Review Figure 16.2
- ▶ As the embryo develops, its cells gradually become determined—committed to developing into particular parts of the embryo and particular adult structures. Following determination, cells eventually differentiate into their final, often specialized, forms.

The Role of Differential Gene Expression in Cell Differentiation

- ▶ The zygote is totipotent; it contains the entire genetic constitution of the organism and is capable of forming all adult tissues.
- ▶ Two lines of evidence show that differentiation does not involve permanent changes in the genome. First, nuclear transplant and cloning experiments show that the nucleus of a differentiated cell retains the ability to act like a zygote nucleus and stimulate the production of an entire organism. Second, molecular investigations have shown directly that all cells contain all genes for the organism, but that only certain genes are expressed in a given tissue. Review Figures 16.3, 16.4, 16.5
- ▶ Embryonic stem cells are totipotent, and can be cultured in the laboratory. With suitable environmental stimulation, these cells can be induced to form cells that differentiate. Review Figure 16.6

The Role of Polarity in Cell Determination

- ▶ Unequal distribution of cytoplasmic determinants in the egg, zygote, or embryo leads to cell determination in normal development. Experimentally altering this distribution can alter gene expression and produce abnormal or nonfunctional organisms. Review Figure 16.7

310 CHAPTER SIXTEEN

The Role of Embryonic Induction in Cell Determination

- ▶ Some embryonic animal tissues direct the development of their neighbors by secreting inducers.
- ▶ Induction is often reciprocal: One tissue induces a neighbor to change, and the neighbor, in turn, induces the first tissue to change, as in eye formation in vertebrate embryos. Review Figure 16.8
- ▶ The nematode *Caenorhabditis elegans* provides a striking example of induction. The adult consists of 959 cells that develop from the fertilized egg by a precise pattern of cell divisions and other events. Review Figure 16.9
- ▶ Induction in *C. elegans* can be very precise, with individual cells producing specific effects in just two or three neighboring cells. Review Figure 16.9

The Role of Pattern Formation in Organ Development

- ▶ In plants and animals, programmed cell death (apoptosis) is important in pattern formation. Some genes whose protein products regulate apoptosis have been identified. Review Figure 16.10
- ▶ Plants have organ identity genes that interact to cause the formation of sepals, petals, stamens, and carpels. Mutations of these genes may cause undifferentiated cells to form a different organ. Review Figures 16.11, 16.12
- ▶ Both plants and animals use positional information as a basis for pattern formation. Gradients of morphogens provide this information.

The Role of Differential Gene Expression in Establishing Body Segmentation

- ▶ The fruit fly *Drosophila melanogaster* has provided much information about the development of body segmentation. Some of this information applies to other animals.
- ▶ The first genes to act in determining *Drosophila* segmentation are maternal effect genes, such as bicoid and nanos, which encode morphogens that form gradients in the egg. These morphogens act on segmentation genes to define the anterior-posterior organization of the embryo. Review Figures 16.13, 16.14, 16.15

► Segmentation develops as the result of a transcriptionally controlled cascade, with the product of one gene promoting or repressing the expression of another gene. There are three kinds of segmentation genes, each responsible for a different step in segmentation. Gap genes organize large areas along the anterior-posterior axis, pair rule genes divide the axis into pairs of segments, and segment polarity genes see to it that each segment has an appropriate anterior-posterior axis. Review Figure 16.14

► The Bicoid and Nanos proteins act as a transcription factor and translation regulator, respectively, to control the level of expression of gap genes. Gap genes encode transcription fac-

tors that regulate the expression of pair rule genes. The products of the pair rule genes are transcription factors that regulate the segment polarity genes.

► Activation of the segmentation genes leads to the activation of the appropriate homeotic genes in different segments. The homeotic genes define the functional characteristics of the segments.

► Mutations in homeotic genes often have bizarre effects, causing structures to form in inappropriate parts of the body. Review Figure 16.16

► Homeotic genes contain the homeobox, which encodes an amino acid sequence that is part of many transcription factors. Review Figure 16.17

Evolution and Development

► The homeobox is found in key genes of distantly related species; thus numerous regulatory mechanisms may trace back to a single evolutionary precursor. Review Figure 16.18

► The activities of similar genes result in similar programs of development in different organisms. These genes include homeotic genes, genes involved in eye formation, and genes that determine dorsal and ventral surfaces. Review Figure 16.19

For Discussion

1. Molecular biologists can insert genes attached to high-level promoters into cells (see Chapter 16). What would happen if the following were inserted and overexpressed? Explain your answers.

a. *ced-9* in embryonic nerve cell precursors in *C. elegans*

b. MyoD in undifferentiated myoblasts

c. *nanos* at the anterior end of the *Drosophila* embryo

2. A powerful method to test for the function of a gene in development is to generate a "knockout" organism, in which the gene in question is inactivated. What do you think would happen in each of the following?

a. *C. elegans* with *ced-9* knocked out

b. *Drosophila* with *nanos* knocked out

c. *Drosophila* with *bithorax* knocked out

3. During development, the potential of a tissue becomes ever more limited until, in the normal course of events, the potential is the same as the original fate. On the basis of what you have learned here and in Chapter 14, discuss possible mechanisms for the progressive limitation of developmental potential.

4. How were biologists able to obtain such a complete accounting of all the cells in *C. elegans*? Why can't we reason directly from studies of *C. elegans* to comparable problems in our own species?



Recombinant DNA and Biotechnology



At the beginning of CHAPTER 3, WE INTRO-duced you to spider silk, a family of proteins that show a fascinating combination of strength and elasticity. There is great interest in studying this biomaterial, not only to find out how it meets the structural challenges of the spider's web, but also because it has potential uses to humans, ranging from replacing expensive Kevlar in bulletproof vests to being used for surgical sutures and even to snag airplanes landing on aircraft carriers. The problem with spider silk is one of supply. "Milking" spiders, as has been done for centuries to get silk for fabrics from moth larvae, does not work on a commercial scale.

Because silk is made of a protein, one approach to the mass production of silk has been to use applied biology— biotechnology

—to produce silk proteins in quantity and then develop ways to spin them into fibers, just as the spider does in its abdomen. This process has two parts. First, the gene for the silk protein is isolated from the spider genome. Then, the gene is put into a system that can express it in quantity. Silk genes turn out to be similar to the proteins they code for in that they are composed of repetitive domains. Through cutting and splicing of DNA, silk genes have been inserted into bacteria and into yeast, a relatively simple eukaryote. In both cases, an active promoter region was fused to the silk gene so that it would be expressed. Unfortunately, both of these types of host cells for this new DNA—called recombinant DNA—make insoluble silk that remains inside the cells.

Spider silk glands are similar in structure and function to animal mammary glands, in that both of them have epithelial cells that manufacture and secrete water-soluble, complex proteins in large amounts. Exploiting these similarities, scientists at a biotechnology company inserted the spider silk gene, with an accompanying mammary gland promoter for tissue-specific expression, into a goat, which produced abundant (10 g/L), soluble, easily purified silk in its milk. Creating such a transgenic goat is a tricky procedure, so a reliable silk producer goat has now been cloned, using the method described in the previous chapter. The next step, currently under way, is to develop a way to spin this silk protein into fibers.

A Factory for Spider Silk

These goats belong to a strain that is being genetically engineered to make spider silk in their milk.

This story—from problem to solution, from protein to expressed gene—has been repeated many times in the past two decades. The products of biotechnology range from life-saving drugs that there is no other way to make in adequate amounts to crop plants with improved agricultural characteristics. Although the basic techniques of DNA manipulation have been called revolutionary, most of them come from the knowledge of DNA transcription and translation that we described in earlier chapters.

We begin this chapter with a description of how DNA molecules can be cut into smaller fragments, and the fragments from different sources covalently linked to create recombinant DNA in the test tube. Recombinant (or any other) DNA can be introduced into a suitable prokaryotic or eukaryotic host cell. Sometimes, the purpose of adding new gene(s) to a host cell or organism is to ask an experimental question about the role of that gene, which can be answered by placing it in a new environment. In other instances, the purpose is to coax the host cell to make a new gene product.

Cleaving and Rejoining DNA

Scientists have long realized that the chemical reactions used in living cells for one purpose may be applied in the laboratory for other, novel purposes. Recombinant DNA technology—the manipulation and combination of DNA molecules from different sources—is based on this realization, and on an understanding of the properties of certain enzymes and of DNA itself.



312 CHAPTER SEVENTEEN

As we saw in previous chapters, the nucleic acid base-pairing rules underlie many fundamental processes of molecular biology. The mechanisms of DNA replication, transcription, and translation rely on complementary base pairing. Similarly, all the key techniques in recombinant DNA technology—sequencing, rejoining, amplifying, and locating DNA fragments—make use of the complementary base pairing of A with T (or U) and of G with C.

In this section we will identify some of the numerous naturally occurring enzymes that cleave DNA, help it replicate, and repair it. Many of these enzymes have been isolated and purified, and are now used in the laboratory to manipulate and combine DNA. Then we will see how fragments of DNA can be separated and covalently linked to other fragments.

Restriction endonucleases cleave DNA at specific sequences

All organisms must have mechanisms to deal with their enemies. As we saw in Chapter 13, bacteria are attacked by viruses called bacteriophages that inject their genetic material into the host cell. Some bacteria defend themselves against such invasions by first altering their own DNA and then producing enzymes called restriction endonucleases, which catalyze the cleavage of double-stranded DNA molecules—such as those injected by phages—into smaller, noninfectious fragments (Figure 17.1). The bonds cut are between the 3' hydroxyl of one nucleotide and the 5' phosphate of the next one.

There are many such restriction enzymes, each of which cleaves DNA at a specific site defined by a sequence of bases called a recognition site or restriction site. The DNA of the host cell is not cleaved by its own restriction enzymes, because specific modifying enzymes called methyl-lases add methyl ($-\text{CH}_3$) groups to certain bases at the restriction sites of the host's DNA when it is being replicated. The methylation of the host's bases makes the recognition sequence unrecognizable to the

restriction endonuclease. But the unmethylated phage DNA is efficiently recognized and cleaved.

A specific sequence of bases defines each recognition site. For example, the enzyme EcoRI (named after its source, a strain of the bacterium *E. coli*) cuts DNA only where it encounters the following paired sequence in the DNA double helix:

5'...GAATTC...3' 3'...CTTAAG...5'

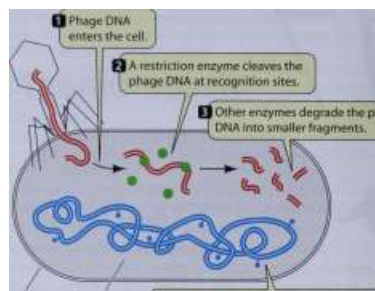
Notice that this sequence reads the same in the 5'-to-3' direction on both strands. It is palindromic, like the word "mom," in the sense that it is the same in both directions from the 5' end. EcoRI has two identical subunits that cleave the two strands between the G and the A.

This recognition sequence occurs on average about once in 4,000 base pairs in a typical prokaryotic genome—or about once per four prokaryotic genes. So EcoRI can chop a large piece of DNA into smaller pieces containing, on average, just a few genes. Using EcoRI in the laboratory to cut

I Phage DNA enters the cell.

IA restriction enzyme cleaves the phage DNA at recognition sites.

Other enzymes degrade the phage DNA into smaller fragments.



Bacterial host cell

Host DNA

O Methyl groups at recognition sites block the restriction endonuclease and protect the bacterial DNA from being cleaved.

17.1 Bacteria Fight Invading Viruses with Restriction Enzymes

Bacteria produce restriction enzymes that cleave and degrade phage DNA. Other enzymes protect the bacteria's own DNA from being cleaved.

small genomes, such as those of viruses that have only a few thousand base pairs, may result in a few fragments. For a huge eukaryotic chromosome with tens of millions of base pairs, the number of fragments will be very large.

Of course, "on average" does not mean that the enzyme cuts all stretches of DNA at regular intervals. The EcoRI recognition sequence does not occur even once in the 40,000 base pairs of the genome of a phage called T7—a fact that is crucial to the survival of this virus, since its host is *E. coli*. Fortunately for the *E. coli* that make EcoRI, the DNA of other phages does contain the recognition sequence.

Hundreds of restriction enzymes have been purified from various microorganisms. In the test tube, different restriction enzymes that recognize different recognition sequences can be used to cut the same sample of DNA. Thus cutting a sample of DNA in many different, specific places is an easy task, and restriction enzymes can be used as "knives" for genetic "surgery."

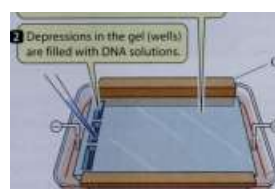
Gel electrophoresis identifies the sizes of DNA fragments

After a laboratory sample of DNA has been cut with a restriction enzyme, the DNA is in fragments, each of which is bounded at its ends by the recognition sequence. As we noted, these fragments are not all the same size, and this property provides a way to separate them from one another. Separating the fragments is necessary to determine the number and sizes (in base pairs) of fragments produced, or to identify and purify an individual fragment of particular interest.

The best way to separate DNA fragments is by gel electrophoresis (Figure 17.2). Because of its phosphate groups,

RESEARCH METHOD

A gel is made up of agarose polymer suspended in a buffer. It sits in a chamber between two electrodes.



Gel support

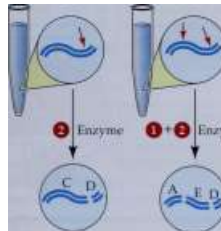
Buffer solution

DNA

solution 9==S)



Enzyme



Enzymes

| Restriction enzyme 1 cuts the DNA once, resulting in fragments A and B.

| Restriction enzyme 2 cuts the DNA once, at a sequence different than the one cut by enzyme 1.

| If both restriction enzymes are used, two cuts are made in the DNA.

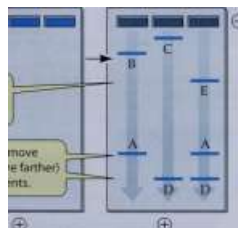
f

O o o+o

Q Each sample is Uj ^^^ [BBgEJ loaded into one well in the gel.

Q Fragments of DNA move toward the positive electrode.

Shorter fragments move faster (and therefore farther) than longer fragments.



77.2 Separating Fragments of DNA by Gel Electrophoresis

A mixture of DNA fragments is placed in a gel and an electric field is applied across the gel. The negatively charged DNA moves toward the positive end of the field, with smaller molecules moving faster than larger ones. When the electric power is shut off, the separate fragments can be analyzed.



DNA is negatively charged at neutral pH. A mixture of DNA fragments is placed in a porous gel, and an electric field (with positive and negative ends) is applied across the gel. Because opposite charges attract, the DNA moves toward the positive end of the field. Since the porous gel acts as a sieve, the smaller molecules move faster than the larger ones. After a fixed time, and while all fragments are still on the gel, the electric power is shut off. The separated fragments can then be examined or removed individually.

Different samples of fragmented DNA can be analyzed side by side on a gel. DNA fragments of known molecular size are often run in a lane on the gel next to the sample to provide a size reference. The separated DNA fragments can be visualized by staining them with a dye that fluoresces under ultraviolet light. Or a specific DNA sequence can be located by denaturing the DNA in the gel, affixing the denatured DNA to a nylon membrane to make a "blot" of the gel, and exposing the fragments to a single-stranded DNA probe with a sequence complementary to the one that is being sought (Figure 17.3). The probe can be labeled in some way—for example, with radioactive phosphorus (P^{32}). Therefore, after hybridization, the presence of radioactivity on the membrane indicates that the probe has hybridized to its target at that location. The gel region containing a desired fragment can be removed when the gel is sliced, and then the pure DNA fragment can be removed from the gel.

Recombinant DNA can be made in a test tube

Some restriction enzymes cut the DNA backbone cleanly, cutting both strands exactly opposite one another. Others make two staggered cuts, cutting one strand of the double helix several bases away from where they cut the other. Fragments cut in this manner are particularly useful in biotechnology.

EcoRI, for example, cuts DNA within its recognition sequence in a staggered manner, as shown at the top of Figure 17.4. After the two cuts in the opposing strands are made, the strands are held together only by the hydrogen bonding between four base pairs. The hydrogen bonds of these few base pairs are too weak to persist at warm temperatures (above room temperature), so the two strands of DNA separate, or denature. As a result, there are single-stranded

314 CHAPTER SEVENTEEN

77.3 Analyzing DNA Fragments

A hybridization probe can be used to locate a specific DNA fragment on an electrophoresis gel.

"tails" at the location of each cut. These tails are called sticky ends because they have a specific base sequence that can bind by base pairing with complementary sticky ends. If more than one recognition site for a given restriction enzyme is present in a DNA sample, numerous fragments can be made, all with the same sequence at their sticky ends.

After a DNA molecule has been cut with a restriction enzyme, the complementary sticky ends can form hydrogen bonds with one another. The original ends may rejoin, or an end may pair with a complementary end from another fragment. Furthermore, because all EcoRI ends are the same, fragments from one source, such as a human, can be joined to fragments from another, such as a bacterium.

When the temperature is lowered, the fragments anneal (come together by hydrogen bonding) at random, but these associations are unstable because they are held together by only a few pairs of hydrogen bonds. The associated sticky ends can be permanently united by a second enzyme, DNA ligase, which forms a covalent bond to "seal" each DNA strand. In the cell, this enzyme unites the Okazaki fragments and mends breaks in DNA (see Chapter 11).

Many restriction enzymes do not produce sticky ends. Instead, they cut both DNA strands at the same base pair within the recognition sequence, making "blunt" ends. DNA ligase can also connect blunt-ended fragments, but it does so with reduced efficiency.

With these two enzyme tools—restriction endonucleases and DNA ligases—scientists can cut and rejoin different DNA molecules to form recombinant DNA (see Figure 17.4). These simple techniques have revolutionized biological science in the past 25 years.

Cloning Genes

The goal of recombinant DNA work is to produce many copies (clones) of a particular gene, either for purposes of analysis or to produce its protein product in quantity. If the DNA is to make its protein, it must be inserted, or transfected, into a host cell. The choice of a host cell—prokaryotic or eukaryotic—is important. Once the host species is selected, the recombinant DNA is brought together with the host cells and, under specific conditions, can enter some of them.

IpAgel is placed in a basic solution that denatures the DNA.

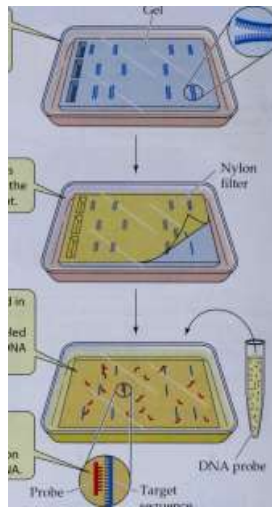
RESEARCH METHOD

Gel

§JA nylon filter picks up the DNA from the gel, creating a blot.

ipThe filter is placed in a solution and a radioactively labeled single-stranded DNA probe is added.

BjThe probe hybridizes to its target sequence on the denatured DNA.



DNA probe

Target sequence

EcoRI cuts at

f

EcoRI has recognition sequences at different points on two DNA strands.

red arrows



CGATCCAGGAATTCATCCAGCC GCTAGGTCCTTAAGTAGGTCGG

AGGCTCTAGAATTCTTCTAGCT TCCGAGATCTTAAGAAGATCGA

t

Q EcoRI digests and separates the DNA strands. The

separated strands have "sticky ends" with unpaired bases.

AATTCATCCAGCC GTAGGTCGG

AATTCTTCTAGCT GAAGATCGA

CGATCCAGG GCTAGGTCCTTAA

AGGCTCTAG TCCGAGATCTTA

7.4 Cutting and Splicing DNA

Some restriction enzymes (EcoRI is shown here) make staggered cuts in DNA. EcoRI can be used to cut DNA from two different sources (shown here in blue and green). At warm temperatures, the two DNA strands will separate (denature), leaving "sticky ends," exposed bases that can hybridize with complementary fragments. When the temperature is lowered, sticky ends from different DNAs can bind to each other, forming recombinant DNA.

CGATCCAGGAATTCTTCTAGCT GCTAGGTCCTTAAGAAGATCGA

! These sticky ends can hydrogen-bond to complementary sticky ends from other DNAs, and the resulting recombinant DNA can be sealed with DNA ligase.

RECOMBINANT DNA AND BIOTECHNOLOGY 315

Because all the host cells proliferate—not just the few that receive the recombinant DNA—the scientist must be able to determine which cells actually contain the sequence of interest. One common method of identifying cells with recombinant DNA is to tag the inserted sequence with genetic markers, called reporter genes, whose phenotypes are easily observed.

Genes can be inserted into prokaryotic or eukaryotic cells

The initial successes of recombinant DNA technology were achieved using bacteria as hosts. As noted in preceding chapters, bacterial cells are easily grown and manipulated in the laboratory. Much of their molecular biology is known, especially for

certain bacteria, such as *E. coli*, and numerous genetic markers can be used to select for cells harboring the recombinant DNA. Bacteria also contain small circular chromosomes called plasmids, which, as we will see, can carry recombinant DNA into the cell.

In some important ways, however, bacteria are not ideal organisms for studying and expressing eukaryotic genes. Bacteria lack the splicing machinery to excise introns from the initial RNA transcript of eukaryotic genes. In addition, many eukaryotic proteins are extensively modified after translation by reactions such as glycosylation and phosphorylation. Often these modifications are essential for the protein's activity. Finally, in some instances, the expression of the new gene in a eukaryote is the point of the experiment. That is, the aim is to produce a transgenic organism, defined as an organism to which a new gene has been added. In these cases, the host for the new DNA may be a mouse, a wheat plant, a yeast, or a human, to name a few examples.

Yeasts, such as *Saccharomyces*, the baker's and brewer's yeasts, are common eukaryotic hosts for recombinant DNA studies. Advantages of using yeasts include rapid cell division (a life cycle completed in 2 to 8 hours), ease of growth in the laboratory, and a relatively small genome size (about 20 million base pairs). The yeast genome is several times larger than that of *E. coli*, yet only 1/150 the size of the mammalian one. Nevertheless, yeast has most of the characteristics of a eukaryote, except for those involved with multicellularity.

Plant cells can also be used as hosts, especially if the desired result is a transgenic plant. The property that makes plant cells good hosts is their totipotency—that is, the ability of a differentiated cell to act like a fertilized egg and produce an entire new organism. Isolated plant cells grown in culture can take up recombinant DNA, and by manipulation of the growth medium, these transgenic cells can be induced to form an entire new plant. The transgenic plant can then be reproduced naturally in the field and will carry and express the gene carried on the recombinant DNA.

Vectors can carry new DNA into host cells

In natural environments, DNA released from one bacterium can sometimes be taken up by another bacterium and genetically transform that bacterium (see Chapter 11),

but this is not common. The challenge of inserting new DNA into a cell is not just getting it into the host cell, but getting it to replicate in the host cell as it divides. As you know, DNA polymerase, the enzyme that catalyzes replication, does not bind to just any sequence of DNA to begin the replication. Rather, like any DNA-binding protein, it recognizes a specific sequence, the origin of replication (see Chapter 11).

There are two general ways in which the newly introduced DNA can become part of a replicon, or replication unit. First, it can insert into the host chromosome after entering the cell. Although this insertion is often a random event, it is nevertheless a common method of integrating a new gene into the host cell. Alternatively, the new DNA can enter the host cell as part of a carrier DNA sequence that already has the appropriate origin of replication. This carrier DNA, targeted at the host cell, is called a vector.

In addition to its ability to replicate independently in the host cell, a vector must have two other properties:

- ▶ A vector must have a recognition sequence for a restriction enzyme, permitting it to form recombinant DNA.
- ▶ A vector must have a genetic marker that will announce its presence in the host cell.

For ease of isolation and manipulation, a vector should also be small in comparison to host chromosomes.

plasmids as vectors. The properties of plasmids make them ideal vectors for the introduction of recombinant DNA into bacteria. Each plasmid is a naturally occurring bacterial chromosome, and has an origin of replication. An *E. coli* plasmid is small, usually 2,000 to 6,000 base pairs, as compared to the main *E. coli* chromosome, which has more than 4 million base pairs. Because it is so small, a plasmid often has only a single site for a given restriction enzyme (Figure 17.5a). This fact is essential because it allows for insertion of new DNA at only that location (see Figure 17.4). When the plasmid is cut with a restriction enzyme, it is transformed into a linear molecule with sticky ends. The sticky ends of another DNA fragment cut with the same restriction enzyme can pair with the sticky ends of the plasmid, resulting in a circular plasmid containing the new DNA.

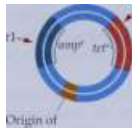
Two other characteristics make plasmids good vectors. First, many plasmids contain genes for enzymes that confer resistance to antibiotics. This characteristic provides a genetic marker for host cells carrying the recombinant plasmid. The second useful property of plasmids is their capacity to replicate independently of the host chromosome. It is not uncommon for a bacterial cell with a single main chromosome to have hundreds of copies of a recombinant plasmid.

The plasmids commonly used as vectors in the laboratory have been extensively altered, and most are combinations of genes and other sequences from several sources. Many of these plasmids have a single marker for antibiotic resistance.

316 CHAPTER SEVENTEEN

(.) Plasmid pBR322 I lost: *E. coli*

PstI



H/i/dlll

■ Br?;»HI

Sfl/1

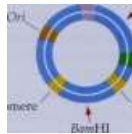
(/) Yeast artificial chromosome Host: yeast

Centromere

EcoRI /

replication (Ori)

| Recognition site for restriction enzymes 5 amp T : Ampicillin resistance gene S tet r : Tetracycline resistance gene



(c) Ti plasmid

Hosts: *Agrobacterium tumefaciens* (plasmid) and infected plants (Ti DNA)

TiDNA

Telomere

Selectable marker

Telomere



Sites for several restriction enzymes

7 7.5 Vectors for Carrying DNA into Cells

(a) A plasmid with genes for antibiotic resistance can be incorporated into an *E. coli* cell, (b) A DNA molecule synthesized in the laboratory becomes a chromosome that can carry its inserted DNA into yeasts. (c) The Ti plasmid, isolated from the bacterium *Agrobacterium tumefaciens*, is an important vector for inserting DNA into many types of plants.

viruses as vectors. Constraints on plasmid replication limit the size of the new DNA that can be spliced into a plasmid to about 5,000 base pairs. Although a prokaryotic gene may be this small, 5,000 base pairs is much smaller than most eukaryotic genes, with their introns and extensive flanking sequences. So, a vector that accommodates larger DNA inserts is needed.

Both prokaryotic and eukaryotic viruses are often used as vectors for eukaryotic DNA. Bacteriophage lambda, which infects *E. coli*, has a DNA genome of more than 45,000 base pairs. If the genes that cause the host cell to die and lyse—about 20,000 base pairs—are eliminated, the virus can still infect a host cell and inject its DNA. The deleted 20,000 base pairs can be replaced with DNA from another organism, thereby creating recombinant viral DNA.

Because viruses infect cells naturally, they offer a great advantage as vectors over plasmids, which often require artificial means to coax them to enter cells. As we will see in Chapter 18, viruses are important vectors for delivering new genes to people in gene therapy.

artificial chromosomes as vectors. Bacterial plasmids are not good vectors for yeast hosts, because prokaryotic and eukaryotic DNA sequences use different origins of replication. Thus a recombinant bacterial plasmid will not replicate in yeast. To remedy this problem, scientists have created in the laboratory a "minimalist chromosome" called the yeast artificial chromosome, or YAC (Figure 17.5b). This DNA molecule contains not only the yeast origin of replication, but sequences for the yeast centromere and telomeres as well, making it a true eukaryotic chromosome. YACs also contain artificially synthesized single restriction sites and useful marker genes (for yeast nutritional requirements). YACs are only about 10,000 base pairs in size, but can accommodate 50,000 to 1.5 million base pairs of inserted DNA.

There has been considerable progress in creating a human artificial chromosome (HAC), which could someday be used as a gene therapy vector. Instead of yeast cen-

tromere and telomere sequences, their human counterparts have been used. The vector acts as a separate minichromosome in human cells, and can be maintained there for months.

plasmid vectors for plants. An important vector for carrying new DNA into many types of plants is a plasmid that is found in *Agrobacterium tumefaciens*. This bacterium lives in the soil and causes a plant disease called crown gall, which is characterized by the presence of growths, or tumors, in the plant. *A. tumefaciens* contains a plasmid called Ti (for tumor-inducing) (Figure 17.5c).

Part of the Ti plasmid is T DNA, a transposon that produces copies of itself in the chromosomes of infected plant cells. The T DNA has recognition sites for restriction enzymes, and new DNA can be spliced into the T DNA region of the plasmid. When the T DNA is thus replaced, the plasmid no longer produces tumors, but the transposon, with the new DNA, is inserted into the host cell's chromosomes. The plant cell containing this DNA can then be grown in culture or induced to form a new, transgenic, plant.

There are many ways to insert recombinant DNA into host cells

Although some vectors, such as viruses, can enter host cells on their own, most vectors require help to do so. A major barrier to DNA entry is that the exterior surface of the plasma membrane, with its phospholipid heads, is negatively charged, as is DNA. The resulting charge repulsion can be alleviated if the exterior of the cells and the DNA are both neutralized with Ca^{2+} (calcium) salts. The salts reduce the charge effect, and the plasma membrane becomes permeable to DNA. In this way, almost any cell, prokaryotic or eukaryotic, can take up a DNA molecule from its environment. In plants and fungi, the cell wall must first be removed by hydrolysis with fungal enzymes; the resulting wall-less plant cells are called protoplasts.

In addition to this "naked" DNA approach, DNA can be introduced into host cells by a variety of mechanical methods:

- ▶ In electroporation, host cells are exposed to rapid pulses of high-voltage current. This treatment temporarily renders the plasma membrane permeable to DNA in the surrounding medium.
- ▶ In injection, a very fine pipette is used to insert DNA into cells. This method is especially useful on large cells such as eggs.
- ▶ In lipofection, DNA is coated with lipid, which allows it to pass through the plasma membrane. For example, DNA can be encased in liposomes, bubbles of lipid that fuse with the membranes of the host cell.
- ▶ In particle bombardment, tiny high-velocity particles of tungsten or gold are coated with DNA and then shot into host cells. This "gene gun" approach must be undertaken with great care to prevent the cell contents from being damaged.

Genetic markers identify host cells that contain recombinant DNA

Even when a population of host cells is allowed to interact with an appropriate vector, only a small percentage of the cells actually take up the vector. Also, since the process of cutting the vector and inserting the new DNA to make recombinant DNA is far from perfect, only a few of the vectors that have moved into the host cells will actually contain the new DNA sequence. How can we select only the host cells that contain the recombinant DNA?

The experiment we are about to describe illustrates an elegant, commonly used approach to this problem. In this example, we use *E. coli* bacteria as hosts and a plasmid vector (see Figure 17.5a) that carries the genes for resistance to the antibiotics ampicillin and tetracycline.

When the plasmid is incubated with the restriction enzyme BamHI, the enzyme encounters its recognition se-

77.6 Marking Recombinant DNA by Inactivating a Gene

Scientists manipulate marker genes within plasmids so they will know which host cells have incorporated the recombinant genes. The host bacteria in this experiment could display any of the phenotypes indicated in the table. Assuming we wish to select only those that have taken up the recombinant plasmid, we can do so by adding antibiotics to the medium surrounding the cells.

quence, GGATCC, only once, at a site within the gene for tetracycline resistance. If foreign DNA is inserted into this restriction site, the presence of these "extra" base pairs within the tetracycline resistance gene inactivates it. So plasmids containing the inserted DNA will carry an intact gene for ampicillin resistance, but not an intact gene for tetracycline resistance. This is the key to the selection of host bacteria that contain the recombinant plasmid (Figure 17.6).

The cutting and splicing process results in three types of DNA, all of which can be taken up by the host bacteria:

- ▶ The recombinant plasmid—the one we want—turns out to be the rarest type of DNA. Its uptake confers on host *E. coli* resistance only to ampicillin.
- ▶ More common are bacteria that take up plasmids that have sealed their own ends back together. These plasmids retain intact genes for resistance to both ampicillin and tetracycline.
- ▶ Even more common are bacteria that take up the foreign DNA sequence alone, without the plasmid; since it is not part of a replicon, it does not survive as the bacteria divide. These host cells will remain susceptible to both antibiotics, as will the vast majority (more than 99.9 percent) of cells that take up no DNA at all.

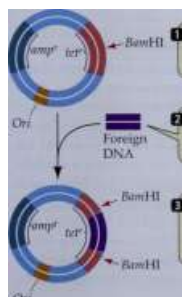
So the unique drug resistance phenotype of the cells with recombinant DNA (tetracycline-sensitive and ampicillin-resistant) marks them in a way that can be detected by simply adding ampicillin and/or tetracycline to the medium surrounding the

cells.

In addition to genes for antibiotic resistance, several other marker genes are used to detect recombinant DNA in host cells. Scientists have created several artificial vectors in the laboratory that include sites for restriction enzymes within the lac operon (see Chapter 13). When this gene is inactivated by the insertion of foreign DNA, the vector no longer carries this operon's function into the host cell. Other reporter genes that have been used in vectors include the gene for luciferase, the enzyme that causes fireflies to glow in the dark; this enzyme causes host cells to glow when supplied with its substrate. Green fluorescent protein, which normally occurs in the jellyfish *Aequopora victo-*

BamHI

BamHI



A plasmid has genes for resistance to both ampicillin (amp^r) and tetracycline (tet^r).

Foreign DNA is inserted at the BamHI recognition site, which is within the tet^r gene.

The resulting recombinant DNA has an intact functional gene for ampicillin resistance but not tetracycline resistance.

CHAPTER SEVENTEEN

RESEARCH METHOD



77.7 A Reporter Gene Announces the Presence of a Vector in Eukaryotic Cells

These cells have taken up a vector that expresses a gene producing green fluorescent protein.

Fluorescein, does not require a substrate to glow, and is now widely used (Figure 17.7).

Many vectors in common use contain only a single marker for antibiotic resistance, outside of the sites for foreign DNA insertion. In this case, the recombinant DNA will have the same antibiotic resistance gene that the non-recombinant plasmid does. The formation of recombinant DNA in the ligase reaction is favored if there is a high concentration of foreign DNA fragments compared to the cut plasmid. So there will be a preponderance of colonies containing recombinant DNA among those that grow in the presence of the antibiotic.

After DNA uptake (or not), host cells are usually first grown on a solid medium. If the concentration of cells dispersed on the solid medium is low, each cell will divide and grow into a distinct colony (see Chapter 13). The colonies that contain recombinant DNA can be identified and removed from the medium, and then grown in large amounts in liquid culture. A quick examination of a plasmid can confirm whether the plasmids in the cells of the colony actually have the recombinant DNA. The power of bacterial transfection to amplify a gene is indicated by the fact that a 1-liter culture of bacteria harboring the human β -globin gene in the pBR322 plasmid has as many copies of the gene as the sum total of all the cells in a typical human being.

Sources of Genes for Cloning

The genes or DNA fragments used in recombinant DNA work are obtained from three principal sources. One source is random pieces of chromosomes maintained as gene libraries. The second source is complementary DNA, obtained by reverse transcription from specific mRNA's. The third source is DNA synthesized by organic chemists in the laboratory. Specific fragments can be deliberately modified to create mutations or to change a mutant sequence back to the wild type.

A DNA sample and plasmids are cleaved with the same restriction endonuclease.

\

i

DNA sample Plasmids

fragments opened

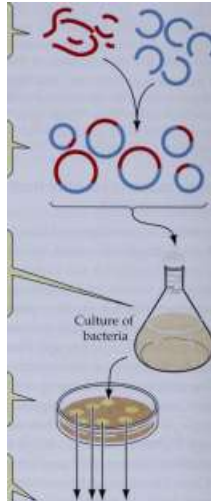
| Fragments and plasmids are mixed and spliced with DNA ligase.

IA mixture of plasmids, all with different inserts, results.

I Bacteria take up the plasmids and are grown in a nutrient medium that selects for recombinant clones.

I Colonies contain clones of each fragment of the original DNA.

I Individual recombinant colonies are isolated and maintained as a pure culture. Each such culture is a "volume" in the gene library.



Individual recombinant colonies (analyzed to determine which genes they contain)

77.8 Constructing a Gene Library

Human chromosomes are broken up into fragments of DNA that are inserted into vectors (plasmids are shown here) and taken up by host bacterial cells, each of which harbors a single fragment of the human DNA. The information in these bacterial colonies constitutes a gene library.

Gene libraries contain pieces of a genome

The 23 pairs of human chromosomes can be thought of as a library that contains the entire genome of our species. Each chromosome, or "volume" in the library, contains, on average, 80 million base pairs of DNA, encoding several thousand genes. Such a huge molecule is not very useful for studying genome organization or for isolating a specific gene. To address this problem, researchers can break each chromosome into smaller pieces using restriction enzymes, and then analyze each piece. These smaller fragments still represent a gene library (Figure 17.8); however, the information is now in many more volumes than 23. Each of the fragments can be inserted into a vector, which can then be

RECOMBINANT DNA AND BIOTECHNOLOGY 319

taken up by a host bacterial cell. Each host cell colony, then, harbors a single fragment of human DNA.

Using plasmids, which are able to insert only a few thousand base pairs of foreign DNA into a bacterium, about a million separate fragments are required to make a library of the human genome. By using phage lambda, which can carry four times as much DNA as a plasmid, the number of volumes is reduced to about 250,000. Although this seems like a large number, a single growth plate can hold up to 80,000 phage colonies, or plaques, and is easily screened for the presence of a particular DNA sequence by denaturing the phage DNA and applying a particular probe for hybridization.

A DNA copy of mRNA can be made

A much smaller DNA library—one that includes only genes transcribed in a particular tissue—can be made from complementary DNA, or cDNA (Figure 17.9). Recall that most eukaryotic mRNA's have a poly A tail—a string of adenine residues at their 3' end (see Figure 14.11). The first step in cDNA production is to extract mRNA from a tissue and allow it to hybridize with a molecule called oligo dT (the "d" indicates deoxyribose), which consists of a string of thymine residues. After the oligo dT hybridizes with the poly A tail of the mRNA, it serves as a primer, and the mRNA as a template, for the enzyme reverse transcriptase, which synthesizes DNA from RNA. In this way, a cDNA strand complementary to the mRNA is formed.

A collection of cDNA's from a particular tissue at a particular time in the life cycle of an organism is called a cDNA library. mRNA's do not last long in the cytoplasm and are often present in small amounts, so a cDNA library is a "snapshot" that preserves the transcription pattern of the cell. cDNA libraries have been invaluable in comparisons of gene expression in different tissues at different stages of development. For example, their use has shown that up to one-third of all the genes of an animal are ex-

f

An mRNA template with a 3' poly A tail is combined with reverse transcriptase enzyme.

©

f

A short oligo dT primer is added and allowed to hybridize with the poly A tail.

Reverse transcriptase synthesizes cDNA using the mRNA template and deoxyribonucleoside triphosphate substrates, creating a DNA-RNA hybrid.

When synthesis is completed, the mRNA is removed, leaving single-stranded cDNA.

pressed only during prenatal development. Complementary DNA is also a good starting point for the cloning of eukaryotic genes. It is especially useful for genes expressed at low levels in only a few cell types.

DNA can be synthesized chemically in the laboratory

When we know the amino acid sequence of a protein, we can obtain the DNA that codes for it by simply making it in the laboratory, using organic chemistry techniques. DNA synthesis has even been automated, and at many institutions, a special service laboratory can make short-to-medium-length sequences overnight for any number of investigators.

How do we design a synthetic gene? Using the genetic code and the known amino acid sequence, we can figure out the most likely base sequence for the gene. With this sequence as a starting point, we can add other sequences, such as codons for translation initiation and termination and flanking sequences for transcription initiation, termination, and regulation. Of course, these noncoding DNA sequences must be the ones actually recognized by the host cell if the synthetic gene is to be transcribed. It does no good to have a prokaryotic promoter sequence near a gene if that gene is to be inserted into a yeast cell for expression. Codon usage is also important: Many amino acids are encoded by more than one codon, and different organisms stress the use of different synonymous codons.

DNA can be mutated in the laboratory

Mutations that occur in nature have been important in proving cause-and-effect relationships in biology. For ex-

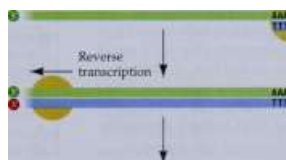
77.9 Synthesizing Complementary DNA

Gene libraries that include only genes transcribed in a particular tissue at a particular time can be made from complementary DNA. cDNA synthesis is especially useful for identifying genes that are present only in a few copies, and is often a starting point for gene cloning.

aaaa... © RNA

I

Reverse transcriptase'



© RNA

© DNA primer

aaaa... © RNA TTTT © C DNA

TTTT © cDNA

\

DNA polymerase uses the cDNA as a template to make a complementary DNA strand.

© ©

©

ample, in Chapter 14, we learned that some people with the disease beta thalassemia have a mutation at the consensus sequence for intron removal and so cannot make proper β -globin mRNA. This example shows the importance of the consensus sequence.

Recombinant DNA technology has allowed us to ask "What if?" questions without having to look for mutations in nature. Because synthetic DNA can be made in any sequence desired, we can manipulate DNA to create specific mutations and then see what happens when the mutant DNA expresses itself in a host cell. Additions, deletions, and base-pair substitutions are all possible with isolated or synthetic DNA.

These mutagenesis techniques have allowed scientists to bypass the search for naturally occurring mutant strains, leading to many cause-and-effect proofs. For example, it was proposed that the signal sequence at the beginning of a secreted protein is essential to its passage through the endoplasmic reticulum membrane. So, a gene coding for such a protein, but with the codons for the signal sequence deleted, was made. Sure enough, when this gene was expressed in yeast cells, the protein did not cross the ER membrane. When the signal sequence codons were added to an unrelated gene encoding a soluble cytoplasmic protein, that protein crossed the ER membrane.

Mutagenesis has also begun to be useful in the design of specific drugs. The advent of a new branch of biology called computational biology has led to sophisticated studies of the three-dimensional shapes and chemical properties of enzymes, substrates, and their possible regulators. Attempts are being made to devise rules to predict the tertiary structure of a protein from its primary structure. For example, if we know the structure of an enzyme, the three-dimensional design of a polypeptide regulating that enzyme might be proposed. Mutant bacterial strains with genes coding for variants of this polypeptide could be made. Then, the variant polypeptides could be isolated and used to test the relationship between structure and activity.

Some Additional Tools for DNA Manipulation

Biological methods are not the only ways of manipulating DNA managed in the laboratory. In Chapter 11, we described DNA sequencing and the polymerase chain reaction, two applications of DNA replication techniques. Here, we examine three additional techniques. One is the use of genetic recombination to create an inactive, or "knocked-out," gene. The second is the use of "DNA chips" to detect the presence of many different sequences simultaneously. The third is the use of antisense RNA to block the translation of specific mRNA's.

Genes can be inactivated by homologous recombination

As we have seen, laboratory-created mutations are an excellent way to ask the "what if" questions about the role of a gene in cell function. Homologous recombination is used to ask these questions at the organism level (Figure 17.10). The

RESEARCH METHOD

Targeted gene

Vector

Vector inserted

f

A marker gene is inserted into the targeted gene.

EP The targeted gene is inactivated by insertion of the marker gene.

f

The vector is inserted into a mouse stem cell

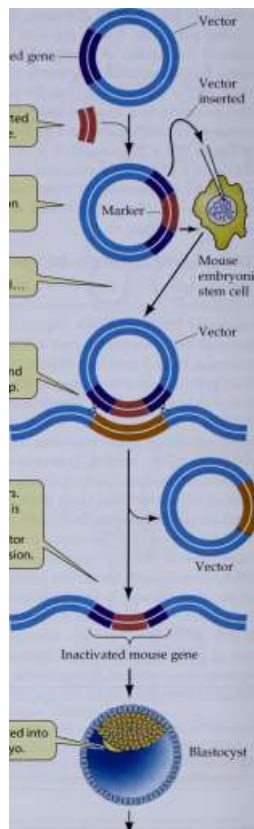
Q ...where the targeted genes on the vector and mouse genome line up.

Mouse gene

Recombination occurs. The inactivated gene is now in the mouse genome, and the vector is lost during cell division.

f

The stem cell is inserted into an early mouse embryo.



Blastocyst

Q A resulting mouse is examined for consequences of carrying an inactivated gene.

T Development " of embryo am

of embryo and birth

T



17.10 Making a Knockout Mouse

Homologous recombination is used to replace a normal mouse gene with an inactivated copy, thus "knocking out" the active gene. Discovering what happens to a mouse with an inactive gene tells us much about the role of that gene.

aim of this technique is to replace a gene inside a cell with an inactivated form of that gene, and then see what happens when the inactive gene is part of an organism. Such a manipulation is called a knockout experiment.

Mice are frequently used in knockout experiments. The mouse gene to be tested is inserted into a plasmid. Restriction enzymes are then used to insert another fragment, containing a genetic marker, in the middle of this gene. Addition of extra DNA to the gene creates havoc with its transcription and translation; a functional mRNA is seldom made from such an interrupted gene. Next, the plasmid is transfected into a mouse embryonic stem cell (see Chapter 16). Because much of the targeted gene is still present in the plasmid (although in two separated regions), there is DNA sequence recognition between the gene on the plasmid and the homologous gene in the mouse genome. As in prophase I of meiosis, the plasmid and the mouse chromosomes line up, and, sometimes, a genetic exchange occurs in which the plasmid's inactive gene is swapped for the functional gene in the host cell. The genetic marker in the insert is used to identify those stem cells carrying the inactivated gene. The transfected stem cell is now inserted into an early mouse embryo, and through some clever tricks, a knockout mouse carrying the inactivated gene in homozygous form can be produced. The phenotype of the mutant mouse is an indication of the role of the gene in the normal, wild-type animal. The knockout technique has been very important in assessing the roles of genes during development.

DNA chips can reveal DNA mutations and RNA expression

The emerging science of genomics deals with two major quantitative circumstances: First, there are a large number of genes in eukaryotic genomes. Second, the pattern of gene expression in different tissues at different times is quite distinctive. For example, a skin cancer cell at its early stage may have a unique mRNA "fingerprint" that differs from that of both normal skin cells and more advanced skin cancer cells.

To find these patterns, scientists could isolate total cell mRNA and test it by hybridization with each gene in the genome, one

gene at a time. But it would be far better to do these hybridizations all in one step. For this, one needs some way to arrange all the DNA sequences in a genome in an array on some solid support.

DNA chip technology has been developed to provide these large arrays of sequences for hybridization. The chips were developed by modifications of methods that have been used for several decades in the semiconductor industry. You may be familiar with the silicon microchip, in which an array of microscopic electric circuits is etched onto a tiny chip. In the same way, DNA chips are glass slides onto which are attached, in precise order, pre-established sequences of DNA (Figure 17.11). Typically, the slide is divided into 24×24 (iM squares, each of which contains about 10 million copies of a particular sequence, up to 20 nucleotides long. A computer controls the addition of nucleotides in a predetermined pattern. Up to 60,000 different sequences can be put on a single chip.

Tissue A Tissue B

T

mRNA is isolated from the tissues.

I cDNA is made from the mRNA's. The two cDNAs are labeled with dyes that can fluoresce different colors.

| The cDNA's are hybridized to the target DNA's on the chip.

Each spot on the array has thousands of copies of a DNA target.

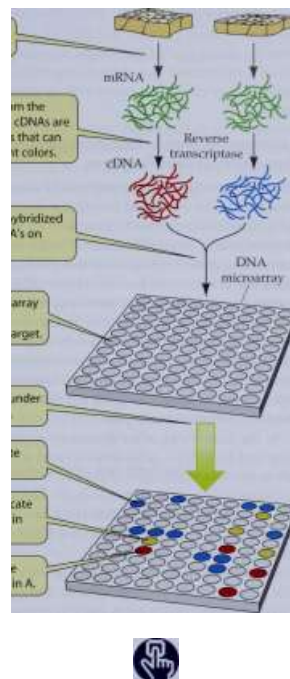
f

The chip is read under fluorescent light.

Blue spots indicate expression in B.

Yellow spots indicate equal expression in A and B.

Red spots indicate equal expression in A



17.11 DNA on a Chip

Thousands of different DNA probes of known sequence can be attached to a glass slide.

If cellular mRNA is to be analyzed, it is usually incubated with reverse transcriptase (RT) to make cDNA (see Figure 17.9), and the cDNA is amplified by PCR prior to hybridization. This technique is called "RT-PCR," and it ensures that mRNA sequences present in only a few copies (or in a small sample such as a cancer biopsy) will be numerous enough to form a signal when hybridized. The amplified cDNA's are coupled to a fluorescent dye and then allowed to hybridize with DNA on a chip. Those DNA sequences that form a hybrid can be located by a sensitive scanner. With the number of genes on a chip approaching that of the largest genomes, these chips will result in an information explosion on mRNA transcription patterns in cells in different physiological states.

Another use for DNA chips is in detecting genetic variants. Suppose one wants to find out if a particular gene, which is 5,500 base pairs long, has any mutations in a particular individual. One way would be to sequence the en-

tire gene, but this would be difficult to do, and would require a large tissue sample. On the other hand, chip technology can be used to make 20-nucleotide fragments along the gene in every possible mutant sequence. Then, probing with the individual's DNA might reveal a particular mutation if it hybridized to a mutant sequence on the chip. This method may provide a rapid way to detect mutations in people.

Antisense RNA and ribozymes can prevent the expression of specific genes

The base-pairing rules not only can be used to make genes; they can also be employed to stop the translation of mRNA. As is often the case, this technique is an example of scientists imitating nature. In normal cells, a rare method of controlling gene expression is the production of an RNA molecule that is complementary to mRNA. This complementary molecule is called antisense RNA because it binds by base pairing to the "sense" bases on the mRNA that code for a protein. The formation of a double-stranded RNA hybrid prevents tRNA from binding to the mRNA, and the hybrid tends to be broken down rapidly in the cytoplasm. So, although the gene continues to be transcribed, translation does not take place.

In the laboratory, after determining the sequence of a gene and its mRNA, scientists can add antisense RNA to a cell to prevent translation of the mRNA (Figure 17.12). The antisense RNA can be added as itself—RNA can be inserted into cells in the same way that DNA is—or it can be made in the cell by transcription from a DNA molecule introduced as a part of a vector.

Without this technique, repressing the synthesis of a specific protein would be very difficult. It is especially useful if a tissue-specific promoter is used to prime transcription of

DNA

f

DNA is transcribed and]_ processed to mRNA. r

Q Antisense RNA is [complementary to mRNA.

mRNA

Tnnnnnnnnnn

i

mRNA

foo^

Antisense RNA hybridizes to the mRNA, blocking its translation.

I ||||| ||| || ||||| 1111111111

|||||||

Antisense RNA

7 7.72 Using Antisense RNA to Block Translation of an mRNA

Once a gene's sequence is determined in the laboratory, the synthesis of its protein can be prevented using antisense RNA that is complementary to its mRNA.

the antisense RNA, so that its expression occurs only in a targeted tissue. An even more effective way to ensure that antisense RNA works is to couple the antisense sequence to a special RNA sequence—a ribozyme—that catalyzes the cleavage of its target RNA.

Antisense RNA (with or without a ribozyme) has been widely used to test cause-and-effect relationships. For example, when antisense RNA was used to block the synthesis of a protein essential for the growth of cancer cells, the cells reverted to a normal state.

Biotechnology: Applications of DNA Manipulation

Biotechnology is the use of microbial, plant, and animal cells to produce materials useful to people. These products include foods, medicines, and chemicals. We have been making some of them for a long time. For example, the use of yeasts to brew beer and wine dates back at least 8,000 years in human history, and the use of bacterial cultures to make cheese and yogurt is a technique many centuries old.

For a long time, people were not aware of the cellular bases of these biochemical transformations. About 100 years ago, thanks largely to Pasteur's work, it became clear that specific bacteria, yeasts, and other microbes could be used as biological converters to make certain products. Alexander Fleming's discovery that the mold *Penicillium* makes the antibiotic penicillin led to the large-scale commercial culture of microbes to produce antibiotics as well as other useful chemicals. Today, microbes are grown in vast quantities to make much of the industrial-grade alcohol, glycerol, butyric acid, and citric acid that are used by themselves or as starting materials in the manufacture of other products.

In the past, the list of such products was limited to those that were naturally made by microbes. The many products that eukaryotes make, such as hormones and certain enzymes, had to be extracted from those complex organisms. Yields were low, and purification was difficult and costly. All this has changed with the advent of gene cloning. The ability to insert almost any gene into bacteria or yeast, along with methods to induce the gene to make its product, has turned these microbes into versatile factories for important products.

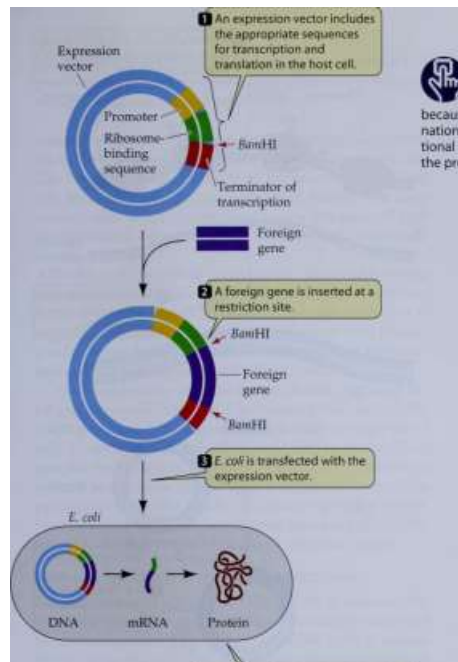
Expression vectors can turn cells into protein factories

If a eukaryotic gene is inserted into a typical plasmid (see Figure 17. 5a) and cloned into *E. coli*, little, if any, of the product of the gene will be made by the host cell. The reason is that the eukaryotic gene lacks the bacterial promoter for RNA polymerase binding, the terminator for transcription, and a special sequence on mRNA for ribosome binding. All of these are necessary for the gene to be expressed and its products synthesized in the bacterial cell.

Expression vectors can be made that have all the characteristics of typical vectors, as well as the extra sequences

Expression vector \

| An expression vector includes the appropriate sequences for transcription and translation in the host cell.



f

The foreign gene is expressed in *E. coli* because the expression vector is present.

RECOMBINANT DNA AND BIOTECHNOLOGY 323

77.73 An Expression Vector Allows a Foreign Gene to Be Expressed in a Host Cell

An inserted eukaryotic gene may not be expressed in *E. coli* because it lacks the necessary bacterial sequences for promotion, termination, and ribosome binding. Expression vectors contain these additional sequences, enabling the eukaryotic protein to be synthesized in the prokaryotic cell.

hormone is added. An enhancer that responds to hormonal stimulation might also be added so that transcription and protein production will occur at very high rates—a goal of obvious importance in the manufacture of an industrial product.

A tissue-specific promoter, which is expressed only in a certain tissue at a certain time, can be used if localized expression in an organism is desired. For example, many seed proteins are expressed only in the plant embryo. So coupling a gene to a seed-specific promoter will allow the gene to be expressed only as a seed protein.

Targeting sequences can be added to the gene in the expression vector so that the protein product is directed to an appropriate destination. For example, in a large vessel containing yeast cells making a protein, it might be useful for the protein to be secreted into the extracellular medium for easier recovery.

Medically useful proteins can be made by DNA technology

Many medically useful products have been made by recombinant DNA technology (Table 17.1), and hundreds more are in various stages of development. We will describe three such products to illustrate the techniques that have been used in their development.

needed for the foreign gene to be expressed in the host cell. For bacteria, these additional sequences include the bacterial promoter, the transcription terminator, and the sequence for ribosome binding (Figure 17.13). For eukaryotes, expression

vectors would include the poly A addition site, transcription factor binding sites, and enhancers. Once these sequences are placed at the appropriate location on the vector, the gene will be expressed in the host cell.

An expression vector can be refined in various ways. An inducible promoter, which responds to a specific signal, can be made part of an expression vector. For example, a specific promoter can be used that responds to hormonal stimulation so that the foreign gene can be induced to transcribe its mRNA when the

1 / 1 Some Medically Useful Products of Biotechnology

PRODUCT

USE

Brain-derived neurotropic factor

Colony-stimulating factor

Erythropoietin

Factor VIII

Growth hormone

Insulin

Platelet-derived growth factor Tissue plasminogen activator

Vaccine proteins: Hepatitis B, herpes, influenza, Lyme disease, meningitis, pertussis, etc.

Stimulates regrowth of brain tissue in

patients with Lou Gehrig's disease Stimulates production of white blood

cells in patients with cancer and AIDS Prevents anemia in patients undergoing

kidney dialysis Replaces clotting factor missing in

patients with hemophilia A Replaces missing hormone in people of

short stature Stimulates glucose uptake from blood in

some people with diabetes Stimulates wound healing Dissolves blood clots after heart attacks

and strokes Prevent and treat infectious diseases

324 CHAPTER SEVENTEEN

tissue plasminogen activator. In most people, when a wound begins bleeding, a blood clot soon forms to stop the flow. Later, as the wound heals, the clot dissolves. How does the blood perform these conflicting functions at the right times? Mammalian blood contains an enzyme called plasmin that catalyzes the dissolution of the clotting proteins. But plasmin is not always active; if it were, a blood clot would dissolve as soon as it formed! Instead, plasmin is "stored" in the blood in an inactive form called plasminogen. The conversion of plasminogen to plasmin is activated by an enzyme appropriately called tissue plasminogen activator (TPA), which is produced by cells lining the blood vessels. Thus, the reaction is

Q Antibody binds to TPA as it is being made on polysomes, allowing TPA

plasminogen (inactive)

TPA

-> plasmin (active)

Heart attacks and many strokes are caused by blood clots that form in important blood vessels leading to the heart or the brain, respectively. During the 1970s, a bacterial enzyme, streptokinase, was found to stimulate the quick dissolution of clots in some patients with these afflictions. Treating people with this enzyme saved lives, but there were side effects. The drug was a protein foreign to the body, so patients' immune systems reacted against it. More important, the drug sometimes prevented clotting throughout the circulatory system, leading to an almost hemophilia-like condition in some patients.

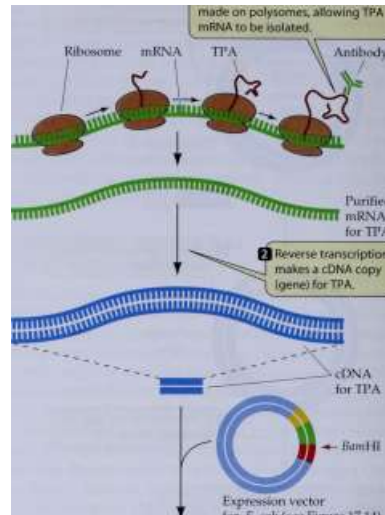
The discovery of TPA and its isolation from human tissues led to the hope that this enzyme could be used to bind specifically to clots, and that it would not provoke an immune reaction. But the amounts of TPA available from human tissues were tiny, certainly not enough to inject at the site of a clot in a patient in the emergency room.

Recombinant DNA technology solved the problem. TPA mRNA was isolated and used to make a cDNA copy, which was then inserted into an expression vector and introduced into *E. coli* (Figure 17.14). The transfected bacteria made the protein in quantity, and it soon became available commercially. This protein has had considerable success in dissolving blood clots in

people undergoing heart attacks and, especially, strokes.

erythropoietin. Another protein made through recombinant DNA methods and widely used in medicine is erythropoietin (EPO). The kidneys produce this hormone, which travels through the blood to the bone marrow, where it stimulates the division of stem cells to produce red blood cells. People who have suffered kidney failure often require a procedure called kidney dialysis to remove toxins from the blood. However, because dialysis also removes EPO, these patients can become severely anemic (depleted of red blood cells).

As with TPA, the amounts of EPO that can be obtained from healthy people to give to people undergoing dialysis are extremely small, but once again, biotechnology has come to the rescue. The gene for EPO was isolated, inserted in an expression vector, and introduced into bacteria. Large

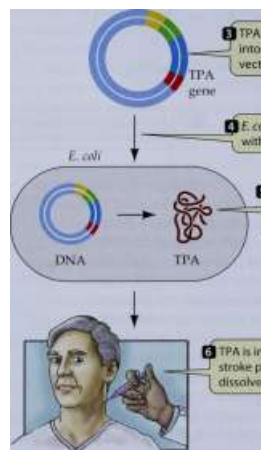


cDNA for TPA

BamHI

Expression vector

for E. coli (see Figure 17.14)



TPA DNA is inserted into the expression vector.

E.coli is transfected with the vector.

TPA protein is made in large amounts.

TPA is injected into a stroke patient to dissolve the blood clot.

17.14 Tissue Plasminogen Activator: From Protein to Gene to Pharmaceutical

TPA is a naturally occurring human protein that prevents blood from clotting. Its isolation and use as a pharmaceutical agent for treating patients suffering from blood clotting in the brain or heart—in other words, strokes and heart attacks—was made possible by recombinant DNA technology.

amounts of the protein are now produced by bacteria and given to tens of thousands of people undergoing dialysis, with great success at reducing anemia.

human insulin. One of the first important medications made by recombinant DNA methods was human insulin. This hormone, normally made by the pancreas, stimulates cells to take up glucose from the blood. People who have certain forms

of diabetes mellitus have a deficiency of pancreatic insulin. Injections of the hormone can compensate for this deficiency.

In the past, the injected insulin was obtained from the pancreases of cows and pigs, which caused two problems. First, animal insulin is laborious to purify; second, it is slightly different in its amino acid sequence from human insulin. Some diabetics' immune systems detect these differences and react against the foreign protein.

The ideal solution is to use human insulin, but until the advent of recombinant DNA technology, it was available only in minuscule amounts. Since insulin is made up of only 51 amino acids, scientists were able to synthesize a gene for this protein in the laboratory. This gene (there were actually two of them, one for each polypeptide chain of the protein) was inserted into *E. coli* via an expression vector. Production of human insulin by the bacteria made widespread use of the human hormone by diabetics feasible.

DNA manipulation is changing agriculture

The cultivation of plants and husbanding of animals that constitute agriculture give us the world's oldest examples of biotechnology, dating back more than 8,000 years in human history. Over the centuries, people have adapted crops and farm animals to their needs. Through cultivation and breeding (artificial selection), desirable characteristics, such as ease of cooking the seeds or quality of the meat, have been imparted and improved. In addition, people have developed crops with desirable growth characteristics, such as a reliable ripening season and resistance to diseases.

Until recently, the most common way to improve crop plants and farm animals was to select and breed varieties with the desired phenotypes that existed in nature through mutational variation. The advent of genetics in the past century was followed by its application to plant and animal breeding. A crop plant or animal with a desirable gene could be identified, and through deliberate crosses, a single gene or, more usually, many genes could be introduced into a widely used variety of that crop.

Despite spectacular successes, such as the breeding of "supercrops" of wheat, rice, and corn, such deliberate crossing remains a hit-or-miss affair. Many desirable characteristics are complex in their genetics, and it is hard to predict accurately the results

of a cross. Moreover, traditional crop plant breeding takes a long time: Many plants can reproduce only once or twice a year—a far cry from the rapid reproduction of bacteria or fruit flies.

Modern recombinant DNA technology has two advantages over traditional methods of breeding. First, the molecular approach allows a breeder to choose specific genes, making the process more precise and less likely to fail as a result of the incorporation of unforeseen genes. The ability to work with cells in the laboratory and then regenerate a whole plant by cloning makes the process much faster than the years needed for traditional breeding. The second advantage—and it is truly an amazing one—is that these molecular methods allow breeders to introduce any gene from any organism into a plant or animal species. This ability, combined with mutagenesis techniques, expands the range of possible new characteristics to an almost limitless horizon.

Biotechnology has found many applications in agriculture (Table 17.2), ranging from improving the nutritional properties of crops, to using animals as gene product factories, to using edible crops to make oral vaccines. We will describe a few examples here to demonstrate the approaches that have been used.

PLANTS THAT MAKE THEIR OWN INSECTICIDES. Humans are

not the only species that consumes crop plants. Plants are subject to infections by viruses, bacteria, and fungi, but probably the most important crop pests are herbivorous insects. From the locusts of biblical (and modern) times to the cotton boll weevil, insects have continually eaten the crops people grow.

The development of insecticides has improved the situation somewhat, but insecticides have their problems. Most, such as the organophosphates, are relatively nonspecific, killing not only the pests in the field but beneficial insects in the ecosystem as well. Some even have toxic effects on other organisms, including people. What's more, insecticides are applied to the surface of crop plants and tend to be blown away to adjacent areas, where they may have unforeseen effects.

326 CHAPTER SEVENTEEN

Some bacteria have solved their own pest problem by producing proteins that kill insect larvae that eat them. For example, there are dozens of strains of *Bacillus thuringiensis*, each of which produces a protein toxic to the insect larvae that prey on it. The toxicity of this protein is 80,000 times that of the usual commercial insecticides. When a hapless larva eats the bacteria, the toxin becomes activated, binding specifically to the insect's gut to produce holes. The insect starves to death.

Dried preparations of *B. thuringiensis* have been sold for decades as a safe, biodegradable insecticide. But biodegradation is their limitation, because it means that the dried bacteria must be applied repeatedly during the growing season. A more permanent approach would be to have the crop plants make the toxin themselves.

The toxin genes from different strains of *B. thuringiensis* have been isolated and cloned. They have been extensively modified by the addition of plant promoters and terminators, plant poly A signals, plant codon usage, and plant regulatory elements on DNA. These modified genes have been introduced into plant cells in the laboratory using the Ti plasmid vector (see Figure 17.5c), and transgenic plants have been grown and tested for insect resistance in the field. So far, transgenic tomato, corn, potato, and cotton crops have been successfully shown to have considerable resistance to their insect predators.

CLONED ANIMALS THAT EXPRESS USEFUL GENES. As we

described in Chapter 16, the cloning of Dolly the sheep was not done only out of scientific curiosity. One of the main objectives of the biotechnology company associated with this experiment is to make useful products in the milk of transgenic dairy animals.

This transgenic strategy is the one that was described in the opening of this chapter for making spider silk. It can also be used to make pharmaceutical products, such as human α -1-antitrypsin (α -LAT). This protein inhibits elastase, an enzyme that breaks down connective tissue. Elastase is found in excess on the surfaces of the lungs of people with cystic fibrosis, and is partly responsible for their severe breathing problems. Thus, using an inhibitor of elastase could alleviate these symptoms in these patients.

The problem is that it has been hard to get enough α -LAT from human serum. To overcome this problem, the gene for human α -LAT was introduced into the fertilized eggs of sheep, next to the promoter for lactoglobulin, a protein made in large amounts in milk (Figure 17.15). The resulting transgenic sheep made large amount of α -LAT in its milk. Since milk is produced in large amounts all year, this natural "bioreactor" produced a large supply of α -LAT, which was easily purified from the other components of the milk.

The production of animals with reliably integrated transgenes is difficult, however, so another approach is to make transgenic clones. In this case, the human gene (with its promoter) is inserted into sheep somatic cells. Those sheep cells that incorporate the transgene can then be used

IUXV

Expression vector has the gene for resistance to the antibiotic, neomycin.

f

Add expression vector to sheep somatic cells.

I Add neomycin, which kills all cells that do not have the vector.

P-lactoglobulin promoter (sheep)

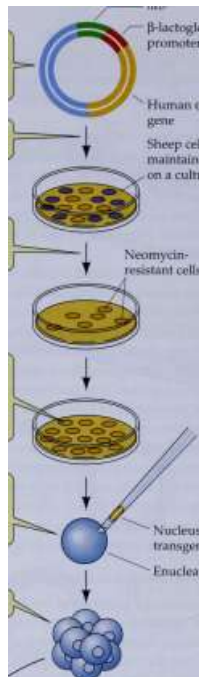
Human α -LAT gene

Sheep cells maintained on a culture dish

I Cells resistant to being killed contain the vector and are transgenic; they grow and divide.

IA transgenic cell is used as nuclear source for fusion with an enucleated egg.

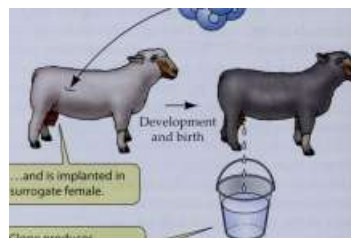
| An embryo develops..



Nucleus from transgenic cell

Enucleated egg

f



...and is implanted in surrogate female.

T

Clone produces abundant α -1 AT in milk,



7.7.5 Production of Transgenic Clones for "Pharming"

The production of transgenic animals involves a combination of DNA technology and reproductive technology.

as the donor nucleus source for cloning (see Figure 16.4). This was the motivation behind the creation of Dolly.

Goats, sheep, and cows are all being used for what has come to be called "pharming," the production of medically useful products in milk. These products include blood clotting factors for treating hemophilia and antibodies for treating colon cancer.

CROPS THAT ARE RESISTANT TO HERBICIDES. Herbivorous

insects are not the only threat to agriculture. Weeds may grow in fields and compete with crop plants for water and soil nutrients. Glyphosate (which is known by the trade name Roundup) is a widely used and effective weed killer, or herbicide. It works only on plants, by inhibiting an enzyme system in the chloroplast that is involved in the synthesis of amino acids. Glyphosate is truly a "miracle herbicide," killing 76 of the world's 78 most prevalent weeds. Unfortunately, it also kills crop plants, so great care must be taken with its use. In fact, it is best used to rid a field of weeds before the crop plant starts to grow. But as any gardener knows, when the crop begins to grow, the weeds reappear. So it would be advantageous if the crop were not affected by the herbicide. Then, the herbicide could be applied to the field at any time, and would kill only the weeds.

Fortunately, some soil bacteria have mutated to develop an enzyme that breaks down glyphosate. Scientists have isolated the gene for this enzyme, cloned it, and added plant sequences for transcription, translation, and targeting to the chloroplast. The gene has been inserted into corn, cotton, and soybean plants, making them resistant to glyphosate. In the late 1990s, this technology expanded so rapidly that half of the U.S. crops of these three plants are now transgenic in this way.

GRAINS WITH IMPROVED NUTRITIONAL CHARACTERISTICS. Hu-

mans must eat foods (or supplements) containing an adequate amount of P-carotene, which the body converts into vitamin A. About 400 million people worldwide suffer from vitamin A deficiency, which makes them susceptible to infections and blindness. One reason is that they eat rice grains, which do not contain P-carotene, but have only a precursor molecule for it. However, other organisms, such as the bacterium *Erwinia* and daffodil plants, have enzymes that can convert the precursor into P-carotene. The genes for this biochemical pathway are present in the bacterial and daffodil genomes, but not in the rice genome.

Scientists isolated two of the genes for the P-carotene pathway from the bacterium and the other two from daffodil plants. They added promoter signals for expression in the developing rice grain, and then added each gene to rice plants by using the vector *Agrobacterium tumefaciens* (see Figure 17.5c). The resulting rice plants produce grains that look yellow because of their high content of P-carotene (Figure 17.16). About 300 grams of this cooked rice a day can supply all the P-carotene a person needs. This new transgenic strain is now being crossed with more locally adapted strains, and it is hoped that the diets of millions of people will be improved soon.

There is public concern about biotechnology

When the initial experiments creating recombinant DNA in the laboratory were done in the 1970s, there was considerable concern, especially by the scientists involved, over the



77.76 Grains From Transgenic Rice Rich in β -Carotene

The grains from this transgenic strain (right) are yellow because they make the pigment (3-carotene, which is converted by humans into vitamin A. Normal rice (left) does not contain P-carotene.

safety of recombinant DNA. After all, the bacterium they used, *E. coli*, normally lives in the human intestine. What would happen if the laboratory strain shared its new genes with the bacteria living in humans? In response to this concern, the scientists involved initially stopped their research, took stock of the implications of what they were doing, and then took elaborate safety precautions to prevent accidental release of the recombinant organisms and their genes. For example, the strains of *E. coli* used in the lab have a number of mutations that make their survival in the human intestine impossible.

As biotechnology developed, it became apparent that these fears for safety were exaggerated. Accidental release of organisms and transfer of genes has not been a problem. Medical products made by DNA technology are widely used and accepted.

However, with the rapid expansion of genetically modified crops, new concerns have been raised. The issue now is a different one in that genetically modified organisms are being designed to be introduced into the natural environment. Indeed, some countries have banned foods that come from genetically modified crops. These concerns are centered on three claims:

► Genetic manipulation is an unnatural interference with nature.

- Genetically altered foods are unsafe to eat.
- Genetically altered plants are dangerous to the environment.

Advocates of biotechnology tend to agree with the first claim. However, they point out that all major crops are unnatural in the sense that they come from highly bred plants growing in a manipulated environment (a farmer's field). The new technology just adds another level of sophistication.

328 CHAPTER SEVENTEEN

DNA

DNA

The concern about safety for humans is countered by the facts that only single genes are added, and that these genes are specific for plant function. For example, the *B. thuringiensis* toxin produced by transgenic plants does not have any effects on people. However, as plant biotechnology moves from adding genes to improve plant growth to adding genes that affect human nutrition, such concerns will become more pressing.

The third concern, about environmental effects, involves the possible "escape" of transgenes from crops to other species. If the gene for herbicide resistance, for example, was inadvertently transferred from a crop to a nearby weed, the latter could thrive in herbicide-treated areas. Or beneficial insects could eat plant materials containing *B. thuringiensis* toxin and die. Transgenic plants undergo extensive field testing before they are approved for use, but the complexity of the biological world makes it impossible to predict all potential environmental effects of transgenic organisms. Because of the potential benefits of agricultural biotechnology (see Table 17.1), scientists believe that it is wise to "proceed with caution."

DNA fingerprinting uses the polymerase chain reaction

"Everyone is unique." This old saying applies not only to human behavior, but also to the human genome. Mutations and recombination through sexual reproduction ensure that each member of a species (except identical twins) has a unique DNA sequence. An individual can be definitively characterized ("fingerprinted") by his or her DNA base sequence.

The ideal way to distinguish an individual from all the other people on Earth would be to describe his or her entire genomic DNA sequence. But since the human genome contains more than 3 billion nucleotides, this idea is clearly not practical. Instead, scientists have looked for genes that are highly polymorphic—that is, genes that have multiple alleles in the human population and are therefore different in different individuals.

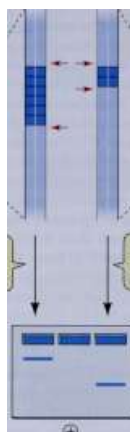
One easily analyzed genetic system consists of short moderately repetitive DNA sequences that occur side by side in the chromosomes. These repeat patterns are inherited. For example, an individual might inherit a chromosome 15 with a short sequence repeated six times from her mother, and the same sequence repeated two times from her father. These repeats, called VNTRs (variable number of tandem repeats), are easily detectable if they lie between two recognition sites for a restriction enzyme. If the DNA from this individual is cut with the restriction enzyme, it will form two different-sized fragments: one larger (the one from the mother) and the other smaller (the one from the father). These patterns are easily seen by use of gel electrophoresis (Figure 17.17). With several different repeated sequences (as many as eight are used, each with numerous alleles), an individual's unique pattern becomes apparent.



Mother's chromosome

There are six repetitive sequences between the two restriction sites.

Gel electrophoresis shows two alleles

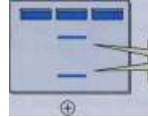




Father's chromosome

There are two repetitive sequences between the two restriction sites.

Gel electrophoresis shows a heterozygote



DNA from the offspring has both 1 parental alleles.

77.77 DNA Fingerprinting

The number of VNTRs inherited by an individual can be used to make a DNA fingerprint.

Typically, these methods require 1 μ g of DNA, or the DNA content of about 100,000 human cells, but this amount is not always available. The power of the polymerase chain reaction (see Figure 11.21) permits the DNA from a single cell to be amplified, producing in a few hours the necessary 1 μ g for restriction and gel analysis.

DNA fingerprints are used in forensics (crime investigation) to help prove the innocence or guilt of a suspect. For example, in a rape case, DNA can be extracted from dried semen or hair left by the attacker and compared with DNA from a suspect. So far, this method has been used to prove innocence (the DNA patterns are different) more often than guilt (the DNA patterns are the same). It is easy to exclude someone on the basis of these tests, but two people could theoretically have the same patterns, since what is being tested is just a small sample of the genome. Therefore, proving that a suspect is guilty cannot rest on DNA fingerprinting alone.

Two fascinating examples demonstrate the use of DNA fingerprinting in the analysis of historical events. Three hundred years of rule by the Romanov dynasty in Russia

ended on July 16, 1918, when Tsar Nicholas II, his wife, and their five children were executed by a firing squad during the Communist revolution. A report that the bodies had been burned to ashes was never questioned until 1989, when a shallow grave with several skeletons was discovered several miles from the presumed execution site. Recent DNA fingerprinting of bone fragments found in this grave indicated that they came from an older man and woman and three female children, who were clearly related to each other (Figure 17.18) and were also related to several living descendants of the Tsar.

The other example involves Thomas Jefferson, the third president of the United States. In 1802, Jefferson was alleged to have fathered a son by his female slave, Sally Hemmings. Jefferson denied this, and his denial was accepted by many historians because of his vocal opposition to mixed-race relationships. But descendants of Hemmings' two oldest sons (the second was named Eston Jefferson) pressed their case. DNA fingerprinting was done using Y chromosome markers from descendants of these two sons as well as the president's paternal uncle (the president had no acknowledged sons). The results show that Thomas Jefferson may have been the father of the second son, but was not the father of the first son.

In addition to such highly publicized cases, there are many other applications of PCR-based DNA fingerprinting. In 1992, the California condor was extinct in the wild. There were only 52 California condors on Earth, all cared for by the San Diego and Los Angeles zoos. Scientists made DNA fingerprints of all these birds so that the geneticists at the zoos could select unrelated individuals for mating in order to increase genetic variation and increase the viability of the offspring. A number of these young birds have now been returned to the wild. A similar program is under way for the threatened Galapagos tortoises (see Chapter 58).

Plant scientists have found in nature or produced by artificial selection thousands of varieties of crops such as rice, wheat, corn, and grapes. The seeds of many of these varieties are kept in cold storage in "seed banks." Samples of these plants are being DNA-fingerprinted to determine which varieties are genetically the same and which are the most diverse, as a guide to future breeding programs.

A related use of PCR is in the diagnosis of infections. In this case, the test shows whether the DNA of an infectious agent is present in a blood or tissue sample. A primer strand matching the pathogen's DNA is added to the sample. If the pathogen is present, its DNA will serve as a template for the primer, and will be amplified. Because so little of the target sequence is needed, and because primers can be made to bind only to a specific viral or bacterial genome, the PCR-based test is extremely sensitive. If an organism is present in small amounts, PCR testing will detect it.

Finally, the isolation and characterization of genes for various human diseases, such as sickle-cell anemia and cystic fibrosis, has made PCR-based genetic testing a reality. We will discuss this subject in depth in the next chapter.



X

These are the parenta genotypes.

STR-1

STR-2 STR-3 STR-4 STR-5 Tsarina Alexandra

15,16

8,8

3,5

12,13

32,36

16,16

7,10

7,7

12,12

11,32

a-

These are the genotypes of three of the children.

u

Tsar Nicholas II

<T6 6 A A

STR-1 STR-2 STR-3 STR-4

STR-5

15,16 8,10 5,7 12,13 11,32

15,16

7,8

5,7

12,13

11,36

15,16 8,10 3,7 12,13 32,36

No remains exist for these two children.

17.18 DNA Fingerprinting the Russian Royal Family

The skeletal remains of Tsar Nicholas II, his wife Alexandra, and three of their children were found in 1989 and subjected to DNA fingerprinting. Five VNTRs were tested. The results can be interpreted as follows. Using the VNTR STR-2 as an example, the parents had genotypes 8,8 (homozygous) and 7,10 (heterozygous). The three children all inherited type 8 from the Tsarina and either type 7 or type 10 from the Tsar.

Chapter Summary

Cleaving and Rejoining DNA

- ▶ Knowledge of DNA transcription, translation, and replication has been used to create recombinant DNA molecules, made up of sequences from different organisms.
- ▶ Restriction enzymes, which are made by microbes as a defense mechanism against viruses, bind to DNA at specific sequences and cut it. Review Figure 17.1
- ▶ DNA fragments generated from cleavage by restriction enzymes can be separated by size using gel electrophoresis. The sequences of these fragments can be further identified by hybridization with a probe. Review Figures 17.2,17.3
- ▶ Many restriction enzymes make staggered cuts in the two strands of DNA, creating "sticky ends" with unpaired bases. The sticky ends can be used to create recombinant DNA if

330 CHAPTER SEVENTEEN

DNA molecules from different species are cut with the same restriction enzyme. Review Figure 17.4

Cloning Genes

- ▶ Bacteria, yeasts, and cultured plant cells are commonly used as hosts for recombinant DNA experiments.
- ▶ Newly introduced DNA must be part of a replicon if it is to be propagated in host cells. One way to make sure that the introduced DNA is part of a replicon is to introduce it as part of a carrier DNA, or vector, that has a replicon.
- ▶ There are specialized vectors to transfect bacteria, yeasts, and plant cells. These vectors must contain a replicon, recognition sequences for restriction enzymes, and genetic markers to identify their presence in the host cells. Review Figure 17.5
- ▶ Naked DNA may be introduced into a host cell by chemical or mechanical means. In this case, the DNA must integrate into the host DNA by itself.
- ▶ When vectors carrying recombinant DNA are incubated with host cells, nutritional, antibiotic resistance, or fluorescent markers can be used to identify which cells contain the vector. Review Figure 17.6

Sources of Genes for Cloning

- ▶ The cutting of DNA by a restriction enzyme produces many fragments that can be individually and randomly combined with a vector and inserted into a host to create a gene library. Review Figure 17.8
- ▶ The mRNA's produced in a certain tissue at a certain time can be extracted and used to create complementary DNA (cDNA) by reverse transcription. This cDNA is then used to make a library. Review Figure 17.9
- ▶ A third source of DNA is synthetic DNA made by chemists in the laboratory. The methods of organic chemistry can be used to create specific, mutated DNA sequences.

Some Additional Tools for DNA Manipulation

- ▶ Homologous recombination can be used to "knock out" a gene in an organism. Review Figure 17.10
- ▶ DNA chip technology permits the screening of thousands of sequences at the same time. Review Figure 17.11
- ▶ An antisense RNA complementary to a specific mRNA can prevent its translation by hybridizing to the mRNA. Review Figure 17.12

Biotechnology: Applications of DNA Manipulation

- ▶ The ability to clone genes has made possible many new applications of biotechnology, such as the large-scale production of eukaryotic gene products.
- ▶ For a vector carrying a gene of interest to be expressed in a host cell, the gene must be adjacent to appropriate sequences for its transcription and translation in the host cell. Review Figure 17.13
- ▶ Recombinant DNA and expression vectors have been used to make medically useful proteins that would otherwise have been difficult to obtain in necessary quantities. Review Figure 17.14, Table 17.1
- ▶ Because plant cells can be cloned to produce adult plants, the introduction of new genes into plants via vectors has been advancing rapidly. The result is crop plants that carry new, useful genes. Review Table 17.2
- ▶ "Pharming" uses transgenic dairy animals that produce useful products in their milk. Review Figure 17.15
- ▶ There is public concern about the applications of biotechnology to food production.

► Because the DNA of an individual is unique, the polymerase chain reaction can be used to identify an organism from a small sample of its cells—that is, to create a DNA fingerprint. Review Figures 17.17, 17.18

For Discussion

1. Compare PCR and cloning as methods to amplify a gene. What are the requirements, benefits, and drawbacks of each method?
2. As specifically as you can, outline the steps you would take to (a) insert and express the gene for a new, nutritious seed protein in wheat; (b) insert and express a gene for a human enzyme in sheep's milk.
3. The *E. coli* plasmid pSCI carries genes for resistance to the antibiotics tetracycline and kanamycin. The *tef* gene has a single restriction site for the enzyme HmdIII. Suppose that both the plasmid and the gene for corn gluten protein are cleaved with HindIII and incubated to create recombinant DNA. The reaction mixture is then incubated with *E. coli* that are sensitive to both antibiotics. What would be the characteristics, with respect to antibiotic sensitivity or resistance, of colonies of *E. coli* containing, in addition to its own genome: (a) no new DNA; (b) native pSCI DNA; (c) recombinant pSCI DNA; (d) corn DNA only? How would you detect these colonies?



Molecular Biology and Medicine



The mother brought her two children to Dr. Asbjorn Foiling in 1934 as a last resort. Since their births, she had watched the conditions of her 6-year-old daughter and 4-year-old son deteriorate. Now both were severely mentally retarded. So far, all of the doctors who had examined the children had expressed sympathy but could do nothing. The mother had noticed a peculiar smell clinging to her children, and she had heard that Dr. Foiling was trained as both a chemist and a physician. Could he help? It turned out he couldn't, because their retardation was irreversible. But while examining these children, Dr. Foiling made a major discovery.

As part of his examination, Dr. Foiling tested the children's urine by adding a brown solution of ferric chloride to look for ketones, which are often excreted by diabetics. This solution normally stays brown, but in diabetics it turns purple. To his surprise, the urine of these children turned the solution dark green. He had never seen this color before, and it was not described in any of his reference books. At first, he suspected that the children were taking a medication that ended up in the urine and reacted with ferric chloride. So he asked the mother to refrain from giving her children any medications for a week and then to bring him two new urine samples. Once again, the samples turned green. Clearly, a substance unique to the bodies of these two children was responsible for the strange color.

Felling's chemistry training served him well. Using analytic chemistry, he purified the substance from the children's urine and identified it as phenylpyruvic acid. Because of the similarity between this substance and the amino acid phenylalanine, Foiling hypothesized that the children were unable to metabolize phenylalanine, and that the excess was being converted to phenylpyruvic acid.

Foiling soon found other mentally retarded people who excreted this substance, and among

Treatment for Phenylketouria

These siblings both suffer from the genetic condition phenylketonuria. The 11-year-old boy was not diagnosed or treated and is severely affected; his younger sister was treated from early infancy with a low-phenylalanine diet; her development and intelligence are normal. Today there is hope of correcting the defect in the gene itself.

the first ten were three pairs of siblings. The parents of these children were mentally normal and did not excrete phenylpyruvic acid. All of these observations fit the idea of an autosomal recessive inherited condition.

Dr. Foiling had discovered the genetic disease phenylketonuria. But it was not the first such disease to be described in biochemical terms. In 1909, Dr. Archibald Garrod had found the cause of alkaptonuria—an inherited disorder in which the patient's urine turns black. Garrod coined the term "inborn errors of metabolism" as a general description of diseases in which genetics and biochemistry are clearly linked. Later, the phenotypes of both phenylketonuria and alkaptonuria were identified as abnormalities of specific enzymes in the same biochemical pathway.

Today the causes of hundreds of such single-gene, single-enzyme diseases are known. In some cases, these discoveries have led to the design of specific therapies and ways to screen for the abnormal proteins in people who do not overtly show the disease. As we will see in this chapter, more precision in describing these abnormalities at the DNA level has come from molecular biology. Even cancer, it turns out, is caused in most cases by abnormalities in genes. The rise of "molecular medicine" is most dramatically shown by undertakings such as gene therapy and the Human Genome Project, which we will discuss at the end of this chapter.



332 CHAPTER EIGHTEEN

Proteins break down
in metabolism

I



CH_n

CH — COOH

I NH₂



Phenylalanine

Q The enzyme that converts phenylalanine to tyrosine is nonfunctional in phenylketonuria (PKU).

OH-



— ^ —

JL

o Because conversion to tyrosine is blocked, phenylalanine and phenylpyruvic acid accumulate in PKU.

CH₃ — C — COOH

O

Phenylpyruvic acid

K

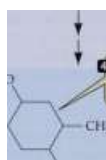
jjj This compound was detected in the urine test with ferric chloride.

CH₃ — CH — COOH

NH-

Tyrosine

HO



Homogentisic acid accumulates in alkaptonuria and turns urine black.

COOH

OH

Homogentisic acid

QTr

The enzyme that catalyzes this conversion is deficient alkaptonuria.

Simpler compounds of normal metabolism

78.7 One Gene, One Enzyme in Humans

Phenylketonuria and alkaptonuria are both caused by abnormalities in a specific enzyme. Knowing the causes of such single-gene, single-enzyme metabolic diseases can aid in the development of screening tests and treatments.

Protein as Phenotype

As we saw in Chapters 11 and 12, genetic mutations are often expressed phenotypically as proteins that differ from the normal wild type. In the first section of this chapter, we identify and discuss the kinds of abnormal proteins that can result from inheritance of an abnormal allele or its origin by mutation. Then we will consider the role of the environment and of patterns of inheritance resulting from autosomal recessives, autosomal dominants, X linkages, and chromosomal abnormalities.

Many genetic diseases result from abnormal or missing proteins

Proteins have many roles in eukaryotic cells, and the genes that code for them can be mutated to cause genetic diseases. Enzymes, receptors, transport proteins, structural pro-

teins, and carriers such as hemoglobin have all been implicated in genetic diseases.

enzymes. Although Dr. Foiling made his discovery in 1934, it was not until 1957 that the complex clinical phenotype of phenylketonuria (PKU) were traced back to its primary phenotype: a single abnormal protein. As Foiling had predicted, phenylalanine hydroxylase, the enzyme that catalyzes the conversion of dietary phenylalanine to tyrosine, was not active in patients' livers (Figure 18.1). Lack of this conversion led to excess phenylalanine in the blood and explained the accumulation of phenylpyruvic acid.

Later, the protein sequences of phenylalanine hydroxylase in normal people were compared with those in individuals suffering from PKU. In many cases, the only difference in the 451 amino acids that constitute this long polypeptide chain was that instead of arginine at position 408, many people with PKU have tryptophan. Once again, the principles of one gene — > one polypeptide and one mutation —> one amino acid change hold true in human diseases as they do in studies of so many other organisms.

How does the abnormality in PKU lead to its clinical symptoms? Since the pigment melanin is made from tyrosine, which patients cannot synthesize but must obtain in the diet, lighter skin and hair color are observed in people with PKU. The exact cause of the mental retardation in PKU remains elusive, but as we will see later in this chapter, it can be prevented.

Hundreds of human genetic diseases that result from enzyme abnormalities have been discovered, many of which lead to mental retardation and premature death. Most of these diseases are rare; PKU, for example, shows up in one newborn infant out of every 12,000. But this is just the tip of the mutational iceberg. Undoubtedly, some mutations result in altered proteins that have no obvious clinical effects. For example, there could be many amino acid changes in phenylalanine hydroxylase that do not affect its catalytic activity.

Analysis of the same protein in different people often shows variations that have no functional significance. In fact, at least 30 percent of all proteins whose sequences are known show detectable amino acid differences among individuals. If one protein variant exists in less than 99 percent of a population (that is, if the protein has another variant at least 1 percent of the time), the protein is said to be polymorphic. The key point is that polymorphism does not necessarily mean disease.

hemoglobin. The first human genetic disease for which an amino acid abnormality was tracked down as the cause was not PKU. It was the blood disease sickle-cell anemia, which most often afflicts people whose ancestors came from

Amino acid position (of 146) 6 7 16 24 26 56 63

95

£

X

c

c

.5 °C re >

M Saskatoon N Baltimore

Tvr

Glu

78.2 Hemoglobin Polymorphism

Only three of the many variants of hemoglobin are known to lead to clinical abnormalities.

the Tropics or from the Mediterranean. Among African-Americans, about 1 in 655 are homozygous for the sickle allele and have the disease. The abnormal allele produces an abnormal protein that leads to sickled red blood cells (see Figure 12.17). These cells tend to block narrow blood capillaries, especially when the oxygen concentration of the blood is low, and the result is tissue damage.

Human hemoglobin is a protein with quaternary structure, containing four globin chains—two α chains and two β chains—as well as the pigment heme (see Figure 3.7). In sickle-cell anemia, one of the 146 amino acids in the β -globin chain is abnormal: At position 6, the normal glutamic acid has been replaced by valine. This replacement changes the charge of the protein (glutamic acid is negatively charged and valine is neutral; see Table 3.2), causing the protein to form long aggregates in the red blood cells. The result is anemia, a deficiency of normal red blood cells.

Because hemoglobin is easy to isolate and study, its variations in the human population have been extensively documented (Figure 18.2). Hundreds of single amino acid alterations in β -globin have been reported. Some of these polymorphisms are even at the same amino acid position. For example, at the same position that is mutated in sickle-cell anemia, the normal glutamic acid may be replaced by lysine, causing hemoglobin C disease. In this case, the anemia is usually not severe. Many alterations of hemoglobin have no effect on the protein's function, and thus no clinical phenotype. This is fortunate, because about 5 percent of all humans are carriers for one of these variants.

receptors and transport proteins. Some of the most common human genetic diseases show their primary phenotype as altered membrane proteins. About one person in 500 is born with familial hypercholesterolemia (FH), in which levels of cholesterol in the blood are several times higher than normal. The excess cholesterol can accumulate on the

MOLECULAR BIOLOGY AND MEDICINE 333

inner walls of blood vessels, leading to complete blockage if a blood clot forms. If a blood clot forms in a major vessel serving the heart, the heart becomes starved of oxygen, and a heart attack results. If a blood clot forms in the brain, the result is a stroke. People with FH often die of heart attacks before the age of 45, and in severe cases, before they are 20 years old.

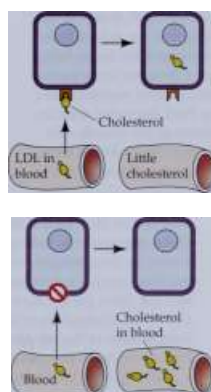
Unlike PKU, which is characterized by the inability to convert phenylalanine to tyrosine, the problem in FH is not an inability to convert cholesterol to other products. People with FH have all the machinery needed to metabolize cholesterol. The problem is that they are unable to transport the cholesterol into the liver cells that use it.

Cholesterol travels in the bloodstream in protein-containing particles called lipoproteins. One type of lipoprotein, low-density lipoprotein, carries cholesterol to the liver cells (Figure 18.3f). After binding to a specific receptor on the membrane of a liver cell, the lipoprotein is taken up by endocytosis and delivers its cholesterol to the interior of the cell. People with FH lack a functional version of

(a) Hypercholesterolemia

Normal liver cell: Cholesterol, as part of low-density lipoprotein (LDL), enters the cell after LDL binds to a receptor.

Familial hypercholesterolemia: Absence of an LDL receptor prevents cholesterol from entering the cells, and it accumulates in the blood.

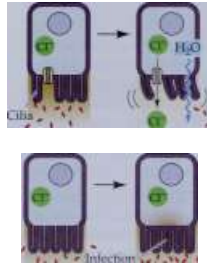


(b) Cystic fibrosis

Normal cell lining the airway: Cl^- leaves the cell through a channel. Water follows by osmosis, and moist thin mucus allows cilia to beat and sweep away foreign particles, including bacteria.

Cystic fibrosis: Lack of a Cl^- channel causes a thick viscous mucus to form. Protective cilia cannot beat properly and remove

bacteria; infections can easily take hold.



- Infection <^ , 'w

Thick mucus

Thin mucus

Thick mucus

Thick mucus

78.3 Genetic Diseases of Membrane Proteins

The left two panels illustrate normal cell function, while the two right panels show the abnormalities caused by {a) hypercholesterolemia and (b) cystic fibrosis.

334 CHAPTER EIGHTEEN

the receptor protein. Of the 840 amino acids that make up the receptor, often only one is abnormal in FH, but this is enough to change its structure so that it cannot bind to the lipoprotein.

Among Caucasians, about one baby in 2,500 is born with cystic fibrosis. The clinical phenotype of this genetic disease is an unusually thick and dry mucus that lines organs such as the tubes that serve the respiratory system. Its dryness prevents cilia on the surfaces of the epithelial cells from working efficiently to clear out the bacteria and fungal spores that we take in with every breath. The results are recurrent and serious infections, as well as liver, pancreatic, and digestive failures. Patients often die in their twenties or thirties.

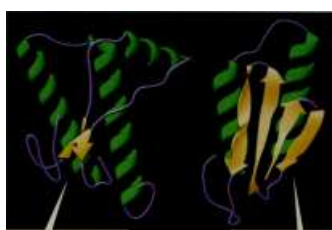
The reason for the thick mucus in patients with cystic fibrosis is a defective version of a membrane protein, the chloride transporter (Figure 18.3E). In normal cells, this membrane channel opens to release Cl^- to the outside of an epithelial cell. The imbalance of Cl^- ions (more are now on the outside of the cell than on the inside) causes water to leave the cell by osmosis, resulting in a moist mucus outside the cell. A single amino acid change in the transporter renders it nonfunctional, which leads to dry mucus and the consequent clinical problems.

structural proteins. About one boy in 3,000 is born with Duchenne's muscular dystrophy. In this genetic disease, the problem is not an enzyme or receptor, but a protein involved in biological structure. People with this disease show progressively weaker muscles and are wheelchair-bound by their teenage years. Patients usually die in their twenties, when the muscles that serve their respiratory system fail. Most people have a protein in their skeletal muscles called dystrophin, which may bind the major muscle protein actin to the plasma membrane of the muscle cells. Patients with Duchenne's muscular dystrophy do not have a working copy of dystrophin, so their muscles do not work.

Coagulation proteins are involved in the clotting of blood at a wound. As we saw in Chapter 17, inactive clotting proteins are always present in the blood, and become active only at a wound. People with the genetic disease hemophilia lack one of the coagulation proteins. Some people with this disease risk death from even minor cuts, since they cannot stop bleeding.

Prion diseases are disorders of protein conformation

Transmissible spongiform encephalopathies (TSE's) are degenerative brain diseases that occur in many mammals, including humans. In these diseases, the brain gradually develops holes, leaving it looking like a sponge. Scrapie, a TSE that causes affected sheep and goats to rub the wool off their bodies, has been known for 250 years. In the 1980s, a TSE appeared in many cows in Britain and was traced to the cows eating products from sheep that had scrapie. Then, in the 1990s, some people who had eaten beef from cows with



Normal prion protein (PrP^c) has many α -helix regions and is relatively soluble.

Abnormal prion protein (PrP^{sc}) has many β -sheet regions and is insoluble.

18.4 Prion Proteins

Normal prion proteins (PrP^c, left) can be converted to the disease-causing form (PrP^{sc}, right), which has a different three-dimensional structure.

TSE got a human version of the disease (dubbed "mad cow disease" by the media), again suggesting that the causative agent could cross species lines.

Another instance of humans consuming an infective agent and getting TSE was kuru, a disease resulting in dementia that occurred among the Fore tribe of New Guinea. In the 1950s, it was discovered that people with kuru had consumed the brains of people who had died of it. When this ritual cannibalism stopped, so did the epidemic of kuru.

Researchers found that TSE's could be transmitted from one animal to another via brain extracts from a diseased animal. But when Tikva Alper treated these extracts with high doses of ultraviolet light to inactivate nucleic acids, they still caused TSE's. She proposed that the causative agent for TSE's was a protein and not a virus, as had been suspected. Later, Stanley Prusiner purified the protein responsible and showed it to be free of DNA or RNA. He called it a proteinaceous infective particle (prion).

Normal brain cells have a membrane protein called PrP^c. A protein with the same amino acid sequence is present in TSE-affected brain tissues, but this protein has an altered shape and is called PrP^{sc} (Figure 18.4). So TSE is not caused by a mutated gene (the primary structures of the two proteins are the same), but is somehow caused by altered protein conformation. The altered three-dimensional structure has profound effects on the protein's function in the cell. Insoluble PrP^{sc} piles up as fibers in brain tissue, causing cell death.

How can the exposure of a normal cell to material containing PrP^{sc} result in a TSE? The abnormal PrP^{sc} protein seems to induce a conformational change in the normal PrP^c protein so that it too becomes abnormal, just as one rotten apple results in a whole barrel full of rotten apples. Just how the conversion occurs and how it causes TSE are unclear.

Most diseases are caused by both heredity and environment

The human diseases for which clinical phenotypes can be traced to a single altered protein may number in the thousands, and in most cases they are dramatic evidence of the one-gene, one-polypeptide principle. Taken together, these diseases have a frequency of about 1 percent in the total population.

Far more common, however, are diseases that are multifactorial; that is, they are caused by many genes and proteins interacting with the environment. Although we tend to call individuals either normal (wild-type) or abnormal (mutant), the sum total of our genes is what determines which of us who eat a high-fat diet will die of a heart attack, or which of us exposed to infectious bacteria will come down with a disease. Estimates suggest that up to 60 percent of all people have diseases that are genetically influenced.

Human genetic diseases have several patterns of inheritance

As in any human genetic system, the alleles that cause diseases are inherited as dominants or recessives, and are carried on autosomes or sex chromosomes (see Chapter 10). In addition, some human diseases are caused by more extensive chromosomal abnormalities.

autosomal recessives. PKU, sickle-cell anemia, and cystic fibrosis are all caused by autosomal recessive mutant alleles. Typically, both parents of an affected person are normal, heterozygous carriers of the abnormal allele. The parents have a 25 percent (one in four) chance of having an affected (homozygous) son or daughter. Because of this low probability and the fact that many families in Western societies now have fewer than four children, it is unusual for more than one child in a family to have an autosomal recessive disease.

In the cells of a person who is homozygous for an autosomal recessive mutant allele, only the nonfunctional, mutant version of the protein it encodes is made. Thus a biochemical pathway or important cell function is disrupted, and disease results. Not unexpectedly, heterozygotes, with one normal and one mutant allele, often have 50 percent of the normal level of functional protein. For example, people who are heterozygous for the allele for PKU have half the number of active molecules of phenylalanine hydroxylase in their liver cells as individuals who carry two normal alleles for this enzyme. But by one mechanism or another, this 50 percent suffices for relatively normal cellular function.

autosomal dominants. An example of a disease caused by abnormal autosomal dominant alleles is familial hypercholesterolemia. In autosomal dominance, the presence of only one mutant allele is enough to produce the clinical phenotype. In people who are heterozygous for familial hypercholesterolemia having half the normal number of functional receptors for low-density lipoprotein on the sur-

face of liver cells is simply not enough to clear cholesterol from the blood. In autosomal dominance, direct transmission from parent to offspring is the rule.

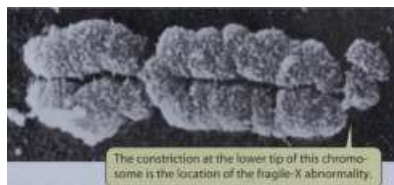
x-linked inheritance. Both hemophilia and Duchenne's muscular dystrophy are inherited as X-linked recessives; that is, the mutant alleles responsible are located on the X chromosome. Thus a son who inherits a mutant allele on the X chromosome from his mother will have the disease, because the Y chromosome does not contain a normal allele. However, a daughter who inherits one mutant allele will be a heterozygous carrier, since she has two X chromosomes, and hence two alleles. Because, until recently, few males with these diseases lived to reproduce, the most common pattern of inheritance has been from

carrier mother to son, and these diseases are much more common in males than in females.

chromosomal abnormalities. Chromosomal abnormalities also cause human diseases. Such abnormalities include an excess or loss of one or two chromosomes (aneuploidy), loss of a piece of a chromosome (deletion), and transfer of a piece of one chromosome to another chromosome (translocation). About one newborn in 200 is born with a chromosomal abnormality. While some of them are inherited, many are the result of meiotic problems such as nondisjunction (see Chapter 9).

Many zygotes that have abnormal chromosomes do not survive development and are spontaneously aborted. Of the 20 percent of pregnancies that are spontaneously aborted during the first 3 months of human development, an estimated half of them have chromosomal aberrations. For example, more than 90 percent of human zygotes that have only one X chromosome and no Y (Turner syndrome) do not live beyond the fourth month of pregnancy.

A common cause of mental retardation is fragile-X syndrome (Figure 18.5). About one male in 1,500 and one female in 2,000 are affected. Near the tip of the abnormal X chromosome is a constriction that tends to break during preparation for microscopy, giving the name for this syndrome. Although the basic pattern of inheritance is that of an X-linked recessive trait, there are departures from this pat-



78.5 A Fragile-X Chromosome at Metaphase

The chromosomal abnormality that causes the mental retardation symptomatic of fragile-X syndrome shows up physically as a constriction.

336 CHAPTER EIGHTEEN

RESEARCH METHOD

(a) Starting with a gene product

t

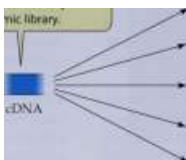
Immature red blood cells contain a lot of β -globin mRNA.



?

β -Globin cDNA is used to probe a human genomic library.

mRNA



This library insert has the β -globin gene.

cDNA

2Sr/ These library inserts do not have the β -globin gene.

-y⁺

(b) Starting with a person who

has a deletion in the chromosome



A boy with Duchenne's muscular dystrophy lacks part of his X chromosome.



These library inserts do not have the dystrophin gene.

This library insert has the ~ 1 dystrophin gene.

The DNA absent in the boy is isolated from the normal X.

The DNA is used to probe a human genomic library.

78.6 Strategies for Isolating Human Genes

(a) Once the sequence for the normal β -globin gene was established by cloning from the isolated protein, it could be compared to the gene sequence of patients with sickle-cell anemia.

(b) When an abnormality is caused by a missing gene, as in Duchenne's muscular dystrophy, researchers can compare the affected chromosome with a normal chromosome and isolate the DNA that is missing, then determine the protein for which this DNA codes.

tern. Not all people with the fragile-X abnormality, however, are mentally retarded; we will describe the reason for this variation later in the chapter.

Mutations and Human Diseases

The isolation and description of human mutations has proceeded rapidly since the development of molecular biology techniques (see Chapter 17). When the primary phenotype was known, as in the case of abnormal hemoglobins, cloning the gene responsible was straightforward, although time-consuming. In other cases, such as Duchenne's muscular dystrophy, a chromosome deletion associated with the disease pointed the way to the missing gene. In still other cases, such as cystic fibrosis, only a subtle molecular marker was available to lead investigators to the gene. In both of the latter examples, the primary phenotype—the defective protein—was unknown; only when the gene was isolated was the protein found.

In the discussions that follow, we will examine how mRNA, chromosome deletions, and DNA markers are used to identify both mutant genes and abnormal proteins involved in genetic diseases. We close this discussion by considering the role of triplet repeats in several genetic diseases.

The logical way to identify a gene is to start with its protein

The primary phenotype for sickle-cell anemia was described in the 1950s as a single amino acid change in β -globin. On the basis of the clinical picture of sickled red blood cells, β -globin was certainly the right protein to examine. By the 1970s, researchers were able to isolate β -globin mRNA from immature red blood cells, which transcribe the globins as their major gene product. A cDNA copy of this mRNA was made and used to probe a human DNA library to find the β -globin gene (Figure 18.6rt). DNA sequencing was then used to compare the normal gene with the gene from patients with sickle-cell anemia, and as previously described, a single-gene mutation was found.

Chromosome deletions can lead to gene and then protein isolation

The inheritance pattern of Duchenne's muscular dystrophy is consistent with an X-linked recessive trait. But until the

late 1980s, neither the abnormal protein involved nor its gene had been described. This failure was not from lack of effort: Almost every muscle protein that could be isolated had been compared for affected people and those without the disease, and had shown no differences.

Then several boys with the disease were found to have a small deletion in their X chromosome. Comparison of the affected chromosomes with normal X chromosomes made possible the isolation of the gene that was missing in the boys (Figure 18.6b).

DNA markers can point the way to important genes

In cases in which no candidate protein or visible chromosome deletion is available to help scientists in isolating a gene responsible for a disease, a technique called positional cloning has been invaluable. To understand this method, imagine an astronaut looking down from space, trying to find her son on a park bench on Chicago's North Shore. Unable to spot the boy with her naked eye, the astronaut picks out landmarks that will lead her to the park. She recognizes the shape of North America, then moves to Lake Michigan, the Sears Tower, and so on. Once she has zeroed in on the North Shore park, she can use advanced optical instruments to find her son.

The reference points for positional cloning are genetic markers on the DNA. These markers can be located within protein-coding regions, within introns, or in spacer DNA between genes. The only requirement is that they be polymorphic, with more than one allele.

As we described in Chapter 17, restriction enzymes cut DNA molecules at specific recognition sequences. On a particular human chromosome, a given restriction enzyme may make hundreds of cuts, producing many DNA fragments that can be probed using gel electrophoresis.

The enzyme EcoRI, for example, cuts DNA at 5'... GAATTC ... 3'. Suppose this recognition site exists in a stretch of human chromosome 7. The enzyme will cut this stretch once and make two fragments of DNA. Now, suppose in some people this sequence is mutated as follows: 5'... GAGTTC ... 3'. This sequence will not be recognized by the restriction enzyme; thus it will remain intact and yield one larger fragment of DNA.

Such DNA differences are called restriction fragment length polymorphisms, or RFLP's (Figure 18.7). A RFLP band pattern is inherited in a Mendelian fashion and can be followed through a pedigree. More than 1,000 such markers have been described for the human genome.

Genetic markers such as RFLP's can be used as landmarks to find genes of interest if they, too, are polymorphic. The key to this method is the well-known observation that if two genes are located near each other on the same chromosome, they are usually passed on together from parent to offspring. The same holds true for any pair of genetic markers.

So, in order to narrow down the location of a gene, a scientist must find a marker and gene that are always inherited together. To do this, family medical histories are taken

RESEARCH METHOD

O⁺ allele:

There is a recognition site for the restriction enzyme, and it cuts the DNA fragment into two.

O⁻ allele:

There is no recognition site for the restriction enzymes, so the DNA is not cut.

bands.

10

100

1000

10000

100000

1000000

10000000

100000000

1000000000



The RFLP is revealed by digestion, electrophoresis, probing, and blotting.

/

Long fragments Short fragments

Type 1 Type 2

homozygote homozygote Heterozygote

(AA) {aa} {Aa}

Aa Aa

^_ ^y\ Q Construct a pedigree.

4 i ±6 ih 14

aa Aa Aa AA Aa aa Aa

23456789

tThisg the m

This gel shows the RFLP patterns of the members of this family.

78.7 RFLP Mapping

Restriction fragment length polymorphisms are differences in DNA sequences that serve as genetic markers. More than 1,000 such markers have been described for the human genome.

and pedigrees are constructed. If the DNA marker and genetic disease are inherited together, then they must be near each other on the chromosome. Unfortunately, "near each other" might be as much as a million base pairs away. The process of locating the gene is thus similar to the astronaut focusing on Chicago: The first landmarks lead to only an approximate location.

How can the gene be isolated? Many sophisticated methods are available for narrowing the search. For example, the neighborhood around the RFLP can be screened for further RFLP's involving other restriction enzymes. With luck, one of them might be linked to the disease-causing gene. Then, DNA fragments from this region can be used to probe for sequences that are expressed and therefore encode proteins. Finally, the candidate gene is sequenced from normal people and from people who have the disease in question. If appropriate mutations are found, the gene of interest has been isolated.

338 CHAPTER EIGHTEEN

18 A

Comparison of Two Genetic Diseases Caused by Point Mutations

VARIABLE

SICKLE-CELL ANEMIA

PHENYLKETONURIA

Protein in phenotype

Length of chain

p-globin

146 amino acids

Phenylalanine hydroxylase 451 amino acids

thymine (Figure 18.8b). However, since the GC pair is now a mismatched GT pair, a different type of repair system comes in and tries to fix the mismatch. Half the time, the mismatch repair system matches a new C to the G, but the other half of the time, it matches a new A to the T, resulting in a mutation.

Larger mutations may involve many base pairs of DNA. For example, some of the deletions in the X chromosome that result in Duchenne's muscular dystrophy cover only part of the dystrophin gene, leading to an incomplete protein and a mild form of the muscle disease. Others cover all of the gene, and thus the protein is missing entirely from muscle, resulting in the severe form of the disease. Still other deletions involve millions of base pairs, and cover not only the dystrophin gene but adjacent genes as well; the result may be several diseases simultaneously.

The isolation of genes responsible for hereditary diseases has led to spectacular advances in the understanding of human biology. Before the genes, and then the proteins, for Duchenne's muscular dystrophy and for cystic fibrosis were isolated, dystrophin and the chloride transporter had never been described. This identification of mutant genes has opened up new vistas in our understanding of how the human body works.

Human gene mutations come in many sizes

Phenylketonuria and sickle-cell anemia are caused by single base-pair point mutations (Table 18.1). As we have seen, some variants of the β -globin gene cause disease, but others do not. Those single base-pair mutations that alter a protein's function usually affect its three-dimensional structure; for example, such a mutation may alter the shape at the active site of an enzyme.

Some mutations lead to not much of a protein at all. For example, some people with cystic fibrosis have a nonsense mutation such that a codon for an amino acid near the beginning of the long chloride transporter protein chain has been changed to a stop codon. Protein translation stops at that point, and a very short peptide is made. As we noted in Chapter 12, other point mutations affect RNA processing, leading to nonfunctional mRNA and no protein synthesis.

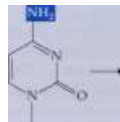
DNA sequencing has revealed that mutations occur most often at certain base pairs. These "hot spots" are often located where cytosine residues have been methylated to 5-methylcytosine (see Chapter 14). This phenomenon is a result of the natural instability of the bases in DNA. Either spontaneously, or with chemical prodding, unmethylated cytosine residues can lose their amino group and form uracil (Figure 18.8a). But the cell nucleus has a repair system that recognizes this uracil as being inappropriate for DNA: After all, uracil occurs only in RNA! So, the uracil is removed and replaced with cytosine.

The fate of 5-methylcytosine that loses its amino group is rather different, since the result of that loss is thymine, a natural base for DNA. The uracil repair system ignores this

(«)

f

When cytosine loses its amino group, uracil is formed.



T

O



NH

Cytosine

GGATCACTC

CCTAGTGAG

"*o Q A DNA repair system

removes this abnormal base and replaces it Uracil ^^ with cytosine.

ggatWac-ccta tgag

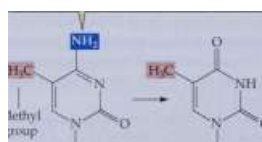
\Gy

GGATCACTC CCTAGTGAG

(b)

?

When 5-methylcytosine loses its amino group, thymine results. Since this is a normal DNA base, it is not repaired.



o When DNA

replicates, half the daughter DNA is mutant...

Methyl group

GGATfIACTC CCTAfITGAG

5-Methylcytosine

GGATCACTC

CCTAGTGAG

Thymine \TWAi

50%,

GGATWACTC Replication _CCJA^rGAG g



GGATCACTC CCTAGTGAG

18.8 5-Methylcytosine in DNA Is a Hot Spot for Mutagenesis

Cytosine can lose an amino group either spontaneously or because of exposure to certain chemical mutagens. The abnormality is usually repaired unless the cytosine residue has been methylated to 5-methylcytosine, in which case a mutation is likely to occur.

MOLECULAR BIOLOGY AND MEDICINE 339

Expanding triplet repeats demonstrate the fragility of some human genes

About one-fifth of all males that have a fragile-X chromosome are phenotypically normal, as are most of their daughters. But many of those daughters' sons are mentally retarded. In a family in which the fragile-X syndrome appears, later generations tend to show earlier onset and more severe symptoms of the disease. It is almost as if the abnormal allele itself is changing—and getting worse. And that's exactly what is happening.

The gene responsible for fragile-X syndrome (FMR1) contains a repeated triplet, CGG, at a certain point in the 5' untranslated region. In normal people, this triplet is repeated 6 to 54 times (average: 29). In the alleles of mentally retarded people with fragile-X syndrome, the CGG sequence is repeated 200 to 1,300 times.

In "premutated" males—those who show no symptoms but have affected offspring—the repeats are fewer— 52 to 200 times. These repeats become more numerous as the daughters of these males pass the chromosome on to their children (Figure 18.9). Expansion to more than 200 repeats leads to the increased methylation of cytosines in the CGG units, accompanied by transcriptional inactivation of the FMR1 gene, which somehow causes mental retardation.

Expanding triplet repeats have been found in over a dozen other diseases, such as myotonic dystrophy (involving repeated CTG triplets) and Huntington's disease (in which CAG is repeated). Many non-disease-causing genes also appear to have these repeats, which can be found within a protein-coding region or outside of it. Because these repeats are so common, they are assumed to play some important role in the genome. How they expand is not known, but may involve DNA polymerase slipping after copying a repeat and then falling back to copy it again.

Genomic imprinting shows that mammals need both a mother and father

Just after fertilization in a mammalian egg, there are two haploid pronuclei—one from the sperm and the other from the egg—in the zygote. They can be distinguished from each other, and they can be removed with a pipette and placed in other eggs. So it is possible to make mouse zygotes in the laboratory with two male or two female pronuclei. These diploid cells should go on to develop into mice, but they don't. Invariably, if the two sets of chromosomes come from only one sex, development begins but is quickly aborted. The same happens in those rare instances when this occurs in humans—for instance, if two sperm enter an empty egg. Again, a fetus never develops.

In addition to showing the obvious need for two sexes, these observations raise the possibility that the male and female genomes are not functionally equivalent. In fact, there are groups of genes that differ in their phenotypic effects depending on which parent they came from. This phenomenon is called genomic imprinting.

Normal allele

6-54 -*-CGG repeats

■MIM.I.M*

L

A normal allele has 6-54 CGG repeats.

Premutated allele

55-200

CGG repeats

h.m-m.h.i.h.i.h.i.h.miii

A

Premutated allele: There are not enough triplets to cause fragile-X syndrome, but they may expand in the next generation.

Defective allele

200-1,300 CGG repeats

Mini um iiiimiw

A

Fragile-X allele: There are many more triplets than normal; this somehow causes mental retardation.

78.9 The CGG Repeat in the Fragile-X Gene Expands with Each Generation

The genetic defect in fragile-X syndrome is caused by excessive repetitions of the CGG triplet.

A dramatic example of genomic imprinting is the inheritance and phenotypic pattern of a certain small deletion on human chromosome 15. If the deletion is on the mother's chromosome 15, the result is a thin child with a wide mouth and prominent jaw (Angelman syndrome). If the deletion is on the father's chromosome 15, the child is short and obese, with small hands and feet (Prader-Willi syndrome). The remaining functional alleles in this region of chromosome 15 must be imprinted in very different ways in the two sexes to result in such different phenotypes. How this happens is not clear.

Detecting Human Genetic Variations

The determination of the precise molecular phenotypes and genotypes for human genetic diseases has had three consequences:

- ▶ Normal cell physiology has been illuminated by studying mutations.
- ▶ Specific biochemical treatments and, potentially, cures have been suggested.
- ▶ Diagnoses may be possible before symptoms first appear, thus making medical intervention possible.

Here we consider the third consequence: the ability to screen for genetic diseases. We will return to the potential for treatments and cures later in the chapter.

340 CHAPTER EIGHTEEN

Genetic screening is the application of a test to identify people who have, are predisposed to, or are carriers of a certain disease. It can be applied at many times of life and used for many purposes. Prenatal testing can identify an embryo or fetus with a disease so that medical intervention can be applied or decisions about continuing the pregnancy can be made. Newborn babies can be tested so that proper medical intervention can be initiated. Asymptomatic people who have a relative with a genetic disease can be tested to determine whether they are carriers. The goal of any screening is not just to provide information; it is to provide information that can be used to reduce an individual's burden resulting from the disease. However, the existence of genetic screening techniques poses ethical questions concerning the uses of this information, as we will see later in the chapter.

Screening for abnormal phenotypes can make use of protein expression

Screening for phenylketonuria in newborns is legally mandatory in many countries, including all of the United States and Canada. Babies who are homozygous for this genetic disease are born with a normal phenotype, because excess phenylalanine in their blood before birth diffuses across the placenta to the mother's circulation. Since the mother is almost always heterozygous, and therefore has adequate phenylalanine hydroxylase activity, her body metabolizes the excess phenylalanine from the fetus. Thus at birth the baby has not yet accumulated abnormal levels of phenylalanine.

After birth, however, the situation changes. The baby begins to consume protein-rich food (milk) and to break down some of its own proteins. Phenylalanine enters the baby's blood, and without the mother's phenylalanine hydroxylase to help, accumulates there. After a few days, the phenylalanine level in the baby's blood may be ten times higher than normal. Within days, the developing brain is damaged, and as Dr. Foiling saw, untreated children with PKU become profoundly mentally retarded.

If PKU is detected early, it can be treated with a special diet low in phenylalanine, and the brain damage avoided. Thus, early detection is imperative. At first, physicians used Felling's ferric chloride test for phenylpyruvic acid in the urine. Unfortunately, babies with PKU do not start excreting large quantities of this substance until they are 4 to 8 weeks old, which can be too late to prevent brain damage. In 1963, Robert Guthrie described a simple screening test for PKU in newborns that today is used almost universally (Figure 18.10). This elegant application of auxotrophic bacteria can be automated so that a screening laboratory can process many samples in a day*

If an infant tests positive for PKU in this screening, he or she must be re-tested using a more accurate chemical

*Guthrie refused to patent the screening test he developed, or to accept any royalties or payment for it. Its immediate and widespread use was at least in part a result of his generosity in allowing the test to be available to oil hospitals at low cost.



A "heel-stick" blood sample is taken a few days after birth.

The sample is dried on blotting paper.

Catalog No. 160-C

Lot No.

BLOOD COLLECTION CARD

Lab Specimen No.

Infant's Name-Infant's Sex

Infant's I.D. No.

Date of Birth/Time

Mother's Name

Hospital Doctor

Date First protein Feeding .

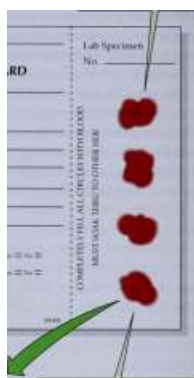
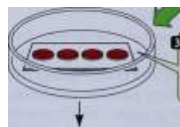
Specimen Date/Tir

LIFE DIAGNOSTICS PO Box 407

Sunderland, MA 01375

Yes = No =

Antibiotics Yes = Ni



The dried spot is cut out and placed on a plate with bacteria that need phenylalanine to grow well.



IA positive test shows a halo of growing bacteria surrounding spots with excess phenylalanine. A negative test shows limited growth.

18.10 Genetic Screening of Newborns for Phenylketonuria

A simple test devised by Robert Guthrie in 1963 is used today to screen newborns for phenylketonuria. Early detection

means that the symptoms of the condition can be prevented by following a therapeutic diet.

assay for phenylalanine. If this test also shows a very high level in the blood, dietary intervention is begun. The whole process—the newborn genetic screen, confirmatory test, diagnosis, and initiation of treatment—must be completed by the end of the second week of life. Since the screening test is inexpensive (about a dollar per test), and since babies with PKU who receive early medical intervention develop practically normally, the benefit of screening is significant.

MOLECULAR BIOLOGY AND MEDICINE 341

Various other conditions are screened for in newborns. The effects of screening on a target population are seen in the example of Tay-Sachs disease, an autosomal recessive disorder that is lethal in childhood. The missing enzyme can be detected with a simple blood test.

Although they do not exhibit any symptoms of the condition, heterozygous carriers of the Tay-Sachs allele are identifiable by their intermediate level of enzyme activity, when compared with that of homozygous wild-type and mutant (affected) persons. If both potential parents know that they carry the mutated allele, the couple can make informed (if difficult) choices about whether to have a child who is likely to be born with a lethal disease.

In the United States, Jewish people of eastern European origin (Ashkenazic Jews) have a Tay-Sachs carrier frequency of 1 in 27, much higher than the overall frequency of 1 in 200 in the general population. Since the 1970s, massive publicity campaigns in the Jewish community have led to widespread screening. As a result, new diagnoses of this disease among Jews have fallen from 65 a year to fewer than 5. In the non-Jewish population, the number of newborns with this disease has remained constant at 15 per year.

There are several ways to screen for abnormal genes

Screening tests based on enzyme activity or protein structure, such as those for PKU and sickle-cell anemia, must be performed on the tissues in which the relevant gene is expressed. For example, the blood level of phenylalanine is an indirect measure of phenylalanine hydroxylase activity in the liver, and hemoglobin electrophoresis shows the presence of sickle (3-globin. But what if blood is difficult to test, as it is in a fetus? What about diseases that are expressed only in the liver or brain and are not reflected in blood? What about proteins whose expression is under cellular control, such that low levels might be the result of a simple dietary factor? Finally, since tissues in heterozygotes often compensate for having just one functional gene by raising the activity of the remaining proteins to near normal levels, how can heterozygotes be identified?

These problems are overcome by DNA testing, which is the most accurate way to test for an abnormal gene. With the description of the genetic mutations responsible for human diseases, it has become possible to directly examine any cell in the body at any time during the life span for mutations. However, these methods work best for diseases caused by only one or a few different mutations. If there are dozens of possible mutations of the gene in the population, simple tests such as the ones we will describe are much less informative.

The polymerase chain reaction (PCR) allows testing of the DNA from even a single cell. Consider, for example, two parents that are both heterozygous for the cystic fibrosis allele, have had a child with the disease, and want a normal child. If the woman is treated with the appropriate hormones, she can be induced to "superovulate," releasing several eggs. One of them can be injected with a single sperm from her husband and the resulting zygote allowed to di-

vide to the 8-cell stage. If one of these cells is removed, it can be tested by PCR for the presence of the cystic fibrosis allele[^]). The remaining 7-cell embryo can be implanted in the mother's womb and go on to develop normally.

Such pre-implantation screening is unusual. More typical are analyses of fetal cells after implantation in the womb. Fetal cells can be analyzed at about the tenth week of pregnancy (by chorionic villus sampling) or during the thirteenth to seventeenth weeks (by amniocentesis). These two sampling methods are described in Chapter 43. In either case, only a few fetal cells are required.

Newborns can also be screened for genetic mutations. The blood samples used for screening for PKU and other disorders contain enough of the baby's blood cells to permit extraction of the DNA, its amplification by PCR, and testing. Pilot studies are under way for testing for sickle-cell disease and cystic fibrosis, and other genes will surely follow.

DNA testing is also now widely used to test adults for heterozygosity. For example, a sister or female cousin of a boy with Duchenne's muscular dystrophy may want to know if she is a carrier of the X chromosome that contains the dystrophin gene mutation. Similarly, the relatives of children with cystic fibrosis can determine their carrier status via DNA testing.

Of the numerous methods of genetic analysis, two are the most widespread. We will describe their use to detect the mutation in the (3-globin gene that results in sickle-cell anemia.

allele-SPECIFIC cleavage differences. There is a difference between the normal and sickle alleles of the (3-globin gene with respect to a restriction enzyme recognition site. Around position 6 in the normal gene is the sequence 5'... CCTGAGGAG... 3'. This sequence is recognized by the restriction enzyme MstII, which will cleave DNA at 5'... CCTNAGG... 3', where "N" is any base. In the sickle mutation (see Table 18.1), the DNA sequence is changed to 5'... CCTGTGGAG... 3'. The single base-pair alteration makes this sequence unrecognizable by MstH. So, when Mst II fails to make the cut in the mutant gene, gel electrophoresis detects a larger DNA fragment (Figure 18.11). This analysis is similar to the use of RFLP's (see Figure 18.7). Thus, the method works only if a restriction enzyme exists that can recognize either the sequence at the mutation or the original sequence that is altered by that mutation.

allele-specific oligonucleotide hybridization. The

allele-specific oligonucleotide hybridization method uses oligonucleotides made in the laboratory that will hybridize either to the denatured normal (3-globin DNA sequence around position 6 or to the sickle mutant sequence. Usually, a probe of at least a dozen bases is needed to form a stable double helix with the target DNA. If the probe is labeled with radioactivity or with a colored or fluorescent substrate, hybridization is readily detected (Figure 18.12). This method is easier and faster than allele-specific cleavage, and will work no matter what the sequence of the normal or mutant allele.

RESEARCH METHOD

Normal Sickle-cell

fji DNA from the normal p-globin allele has a recognition site for the restriction enzyme Msrll at codon position 6.

t

Normal (3-globin DNA is cut into two fragments.

Normal p-globin

T TDNA T

Sickle-coll D\,\

Cut with Msfl

T

DNA from the sickle p-globin allele lacks an MstW recognition site at codon position 6.

tSic an

ckle P-globin DNA is not cut, d a larger fragment results.

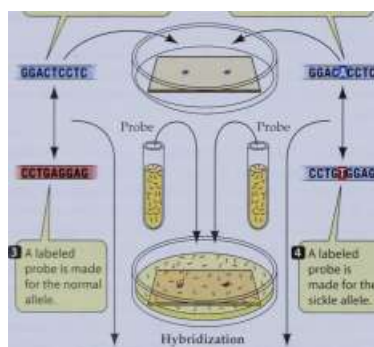
f

The fragments can be identified by gel electrophoresis on the basis of their sizes.

RESEARCH METHOD

Single-stranded DNA is made from the normal p-globin allele (A).

I Single-stranded DNA is made from the sickle p-globin allele (S).



GGACTCCTC CCTGAGGAG

ggac cctc cctgDggag

The red color indicates hybridization.

The blue color indicates lack of hybridization.

Mother

Probe for

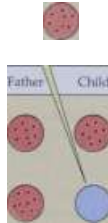
normal allele

Probe for sickle allele



Father

Child



Fetus



t

AS AS AA AS

(Deduced genotypes)

This panel shows results of allele-specific hybridizations for a family.

18.11 DNA Testing by Allele-Specific Cleavage

Allele-specific cleavage, a technique similar to RFLP analysis, can be used to detect mutations such as the one that causes sickle-cell anemia.

Cancer: A Disease of Genetic Changes

Perhaps no malady affecting people in the industrialized world instills more fear than cancer. One in three Americans will have some form of cancer in their lifetime, and at present, one in four will die of it. With a million new cases and half a million deaths in the United States annually, cancer ranks second only to heart disease as a killer. Cancer was less common a century ago; then, as now in many poor regions of the world, people died of infectious diseases and did not live long enough to get cancer. Cancer tends to be a disease of the later years of life; children are less frequently afflicted.

Since the U.S. government declared a "war on cancer" in 1970, a tremendous amount of information on cancer cells—their growth and spread and their molecular changes—has been obtained. Perhaps the most remarkable discovery is that cancer is a disease caused primarily by genetic changes. These changes are mostly alterations in the DNA of somatic cells that are propagated by mitosis.

Cancer cells differ from their normal counterparts

Cancer cells differ from the normal cells from which they originate in two major ways. First, a cancer cell loses control over cell division. Most cells in the body divide only if they are exposed to outside influences, such as growth factors and hormones. Cancer cells do not respond to these controls, and instead divide more or less continuously, ultimately forming tumors (large masses of cells). By the time a

18.12 DNA Testing by Allele-Specific Oligonucleotide Hybridization

Testing of this family reveals that three of them are heterozygous carriers of the sickle-cell allele. The first child, however, has inherited two normal alleles and is neither affected by the disease nor a carrier.

MOLECULAR BIOLOGY AND MEDICINE 343

physician can feel a tumor or see one on an X-ray or CAT scan, it already contains millions of cells.

Benign tumors resemble the tissue they came from, grow slowly, and remain localized where they develop. A lipoma, for example, is a benign tumor of fat cells that may arise in the armpit and remain there. Benign tumors are usually not a problem, but they must be removed if they impinge on an important organ, such as the brain.

Malignant tumors, on the other hand, do not look like their parent tissue at all. A flat, specialized lung epithelial cell in the lung wall may turn into a relatively featureless, round, malignant lung cancer cell. Malignant cells often have irregular structures, such as variable sizes and shapes of nuclei. Many malignant cells express the gene for telomerase and thus do not shorten the ends of their chromosomes after each DNA replication.

The second, and most fearsome, characteristic of cancer cells is their ability to invade surrounding tissues and spread to other parts of the body. This spreading of cancer, called metastasis, occurs in several stages. First, the malignant tumor secretes chemical signals that cause blood vessels to grow to the tumor and supply it with oxygen and nutrients. This process is called angiogenesis. Then, the cancer cells extend into the tissue that surrounds them by actively secreting digestive enzymes to disintegrate the surrounding cells and extracellular materials, working their way toward a blood vessel. Finally, some of the cancer cells enter either the bloodstream or the lymphatic system (Figure 18.13).

The journey of malignant cells through these vessels is perilous, and few of them survive—perhaps one in 10,000. When by chance a cancer cell arrives at an organ suitable for its further growth, it expresses cell surface proteins that allow it to bind to and invade the new host tissue.

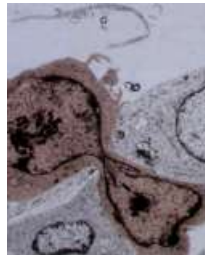
Different forms of cancer affect different parts of the body. About 85 percent of all human tumors are carcinomas —cancers that arise in surface tissues such as the skin

18.2

Human Cancers Known to Be Caused by Viruses



Sn



P '

18.13 The Spread of Cancer

A cancer cell squeezes into a small blood vessel through the vessel's wall. The cancer cell is then carried through the blood and, if it survives the journey, may spread into other tissue.

CANCER

ASSOCIATED VIRUS

Liver cancer Lymphoma,

nasopharyngeal cancer T cell leukemia

Anogenital cancers Kaposi's sarcoma

Hepatitis B virus Epstein-Barr virus

Human T cell

leukemia virus (HTLV-I) Papillomavirus Kaposi's sarcoma

herpesvirus

and the epithelial cells that line the organs. Lung cancer, breast cancer, colon cancer, and liver cancer are all carcinomas. Sarcomas are cancers of tissues such as bone, blood vessels, and muscle. Leukemias and lymphomas affect the cells that give rise to blood cells.

Some cancers are caused by viruses

Peyton Rous's discovery in 1910 that a sarcoma in chickens is caused by a virus that is transmitted from one bird to another spawned an intensive search for cancer-causing viruses in humans. At least five types of human cancer are probably caused by viruses (Table 18.2).

Hepatitis B, a liver disease that affects people all over the world, is caused by the hepatitis B virus, which contaminates blood or is carried from mother to child during birth. The viral infection can be long-lasting and may flare up numerous times. This virus is associated with liver cancer, especially in Asia and Africa, where millions of people are infected. But it does not act to cause cancer by itself. Some gene mutations that are necessary for tumor formation occur in the infected cells of Asians and Africans, although apparently not in Europeans and North Americans.

An important group of virus-caused cancers in North Americans and Europeans are the various anogenital cancers caused by papillomaviruses. The genital and anal warts that these viruses cause often develop into tumors. These viruses seem to be able to act on their own, not needing mutations in the host tissue for tumors to arise. Sexual transmission of these papillomaviruses is unfortunately widespread.

Most cancers are caused by genetic mutations

Worldwide, no more than 15 percent of all cancers may be caused by viruses. What causes the other 85 percent? Because most cancers develop in older people, it is reasonable to assume that one must live long enough for a series of events to occur. This assumption turns out to be correct, and the events are genetic mutations. But these are usually not the germ-line mutations found in genetic diseases. Instead, the mutations in cancer cells are usually somatic mutations—alterations in the genes of non-gamete-producing cells.

DNA can become damaged in many ways. As we have seen, spontaneous mutations arise because of chemical

344 CHAPTER EIGHTEEN

Q A base change may occur spontaneously or be caused by a carcinogen.

o When the top strand replicates, one of two things can happen.

CGGATC GCCTAG

CGoATC GCCTAG

DNA sequence

in >i normal gene



CGGATC GCCTAG

CGQATC GCfTTAG



The changed base may be repaired in a nondividing cell; the gene stays normal...

-==^J an

...or the DNA may replicate

id retain the mutation; the gene is mutated and may cause cancer.

18.14 Dividing Cells Are Especially Susceptible to Genetic Damage and Cancer

A base change is more likely to be repaired in a nondividing cell.

changes in the nucleotides. In addition, certain substances called carcinogens cause mutations that lead to cancer. Familiar carcinogens include the chemicals that are present in tobacco smoke and meat preservatives, ultraviolet light from the sun, and ionizing radiation from sources of radioactivity. Less familiar, but just as harmful, are thousands of chemicals present naturally in the foods people eat. According to one estimate, these "natural" carcinogens account for well over 80 percent of the human exposure to agents that cause cancer.

The common theme in natural and human-made carcinogens is that almost all of them damage DNA, usually by causing changes from one base to another (Figure 18.14). In somatic cells that divide often, such as epithelial and bone marrow cells, there is less time for DNA repair mechanisms to work before replication occurs again. Therefore, such cells are especially susceptible to genetic damage.

Two kinds of genes are changed in many cancers

The changes in the control of cell division that lie at the heart of cancer can be likened to the controls of an automobile. To make a car move, two things must happen: The gas pedal must be pressed, and the brake must be released. In the human genome, some genes act as oncogenes, which act as the "gas pedal" to stimulate cell division, and some as tumor suppressor genes, which "put the brake on" to inhibit it.

oncogenes. The first hint that oncogenes (from the Greek onco-, "mass") were necessary for cells to become cancerous came with the identification of virally induced cancers in animals. In many cases, these viruses bring a new gene into their host cells, one that acts to stimulate cell division when it is expressed in the viral genome. It soon became apparent that these viral oncogenes had counterparts in the genomes of the host cells, called proto-oncogenes, that were not actively transcribed. So the search for genes that are damaged by carcinogens quickly zeroed in on the proto-oncogenes.

Proto-oncogenes are genes that have the capacity to stimulate cell division, but are normally turned "off" in differentiated, nondividing cells. Many are involved in growth factor stimulation (Figure 18.15). Some remarkable proto-oncogenes control apoptosis (programmed cell death). Acti-

vation of these genes by mutation causes them to prevent apoptosis, allowing cells that normally die to continue dividing.

Some proto-oncogenes can be activated by point mutations, others by chromosome changes such as translocations, and still others by gene amplification. Whatever the mechanism, the result is the same: The proto-oncogene becomes activated, and the "gas pedal" for cell division is pressed.

tumor suppressor genes. About 10 percent of all cancer is clearly inherited. Often the inherited form of the cancer is clinically similar to the noninherited form that occurs later in life, called the sporadic form. The major differences are that the inherited form strikes a person much earlier in life and usually shows up as multiple tumors.

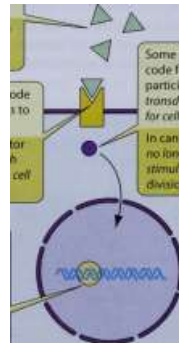
Some proto-oncogenes code for growth factors to stimulate cell division.

In cancer, mutations can cause overproduction of growth factors.

Some proto-oncogenes code for growth factor receptors to stimulate cell division. In cancer, a mutant receptor may no longer need growth factor binding to stimulate cell division.

Some proto-oncogenes code for transcription factors that can activate genes involved in cell division.

In cancer, mutant transcription factors always bind to their target gene promoter.



Some proto-oncogenes code for proteins that participate in the transduction of signals for cell division.

In cancer, mutant proteins no longer need external stimulus, and signal cell division constantly.

18.15 Proto-Oncogene Products Stimulate Cell Division

Mutations can affect any of the several ways proto-oncogenes normally stimulate cell division, thus causing cancer.

(«)

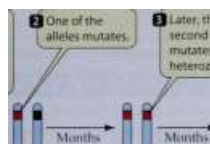
IjMost people are born with two normal alleles for a tumor suppressor gene

| One of the alleles mutates.

fj Later, the second allele mutates (loss of heterozygosity).

ft

Months to years



Months to years

Months to years

Cancer

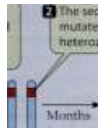
(b)

I Some people inherit one mutated allele of a tumor suppressor gene.

QThe second allele mutates (loss of heterozygosity).

Y

Months to years



Months to years

Cancer

F i

18.16 The "Two-Hit" Hypothesis for Cancer

(a) Although a single mutation can activate a proto-oncogene, two mutations are needed to inactivate a tumor suppressor gene.

(b) An inherited predisposition to cancer occurs in people born with one allele already mutated.

In 1971, Alfred Knudson used these observations to predict that for a tumor to occur, a tumor suppressor gene must be inactivated. But unlike oncogenes, in which one mutated allele is all that is needed for activation, the full inactivation of a tumor suppressor gene requires that both alleles be turned off, which requires two mutational events. It takes a long time for both alleles in a single cell to mutate and cause sporadic cancer. But in inherited cancer, people are born with one mutant allele for the tumor suppressor, and need just one more mutational event for full inactivation of the "brakes" (Figure 18.16).

Various tumor suppressor genes have been isolated and confirm Knudson's "two-hit" hypothesis. Some of these genes are involved in inherited forms of rare childhood cancers such as retinoblastoma (a tumor of the eye) and Wilms' tumor of the kidney, as well as in inherited breast and prostate cancers.

An inherited form of breast cancer demonstrates the effect of tumor suppressor genes. The 9 percent of women who inherit one mutated copy of the gene BRCA1 have a 60 percent chance of having breast cancer by age 50 and an 82 percent chance of developing it by age 70. The comparable figures for women who inherit two normal copies of the gene are 2 percent and 7 percent, respectively.

How do tumor suppressor genes act in the cell? Like the proto-oncogenes, they are normally involved in vital cell functions (Figure 18.17). Some control progress through the cell cycle. The protein encoded by Kb, a gene that was first described for its contribution to retinoblastoma, is active during G1. In the active form, it encodes a protein that binds to and inactivates transcription factors that are necessary for progress to the S phase and the rest of the cell cycle. In nondividing cells, Rb remains active, preventing

MOLECULAR BIOLOGY AND MEDICINE 345

cell division until the proper growth factor signals are present. When the Rb protein is inactivated by mutation, the cell cycle moves forward independent of growth factors.

The protein product of another widespread tumor suppressor gene, p53, also stops cells during G1. It does this by acting as a transcription factor, stimulating the production of (among other things) a protein that blocks the interaction of a cyclin and protein kinase needed for moving the cell cycle beyond G1. This gene is mutated in many types of cancers, including lung cancer and colon cancer.

The pathway from normal cell to cancerous cell is complex

The "gas pedal" and "brake" analogies for oncogenes and tumor suppressor genes, respectively, are elegant but simplified. There are many oncogenes and tumor suppressor genes, some of which act only in certain cells at certain times. Therefore, a sequence of events must occur before a normal cell becomes malignant.

Because colon cancers progress to full malignancy slowly, it is possible to describe the oncogenes and tumor suppressor genes at each stage in great molecular detail. Figure 18.18 outlines the progress of this form of cancer. At least three tumor suppressor genes and one oncogene must be mutated in sequence for an epithelial cell in the colon to become metastatic. Although the occurrence of all these events in a single cell might appear unlikely, remember that the colon has millions of cells, that the cells giving rise to epithelial cells are constantly dividing, and that these changes take

Some tumor suppressor genes code for force/adhesion/recognition proteins.

In cancer, mutations of these genes cause cells to lose adhesion to their neighbors and spread.



J

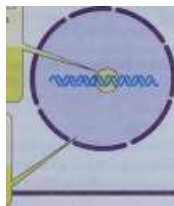
Some tumor suppressor genes code for enzymes involved in DNA repair.

In cancer, mutant proteins no longer repair DNA and mutations accumulate.



Some tumor suppressor genes inhibit cell division by stopping the cell cycle in G₁.

In cancer, mutant proteins no longer block cell division.



18.17 Tumor Suppressor Gene Products Inhibit Cell Division and Cancer

Mutations can affect any of the several ways that tumor suppressor genes inhibit cell division, causing cells to divide and form a tumor.

346 CHAPTER EIGHTEEN

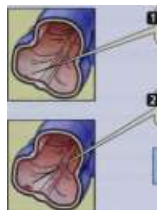


Section through colon (large intestine)

Normal cells

+

Loss of normal tumor suppressor gene APC



A polyp (small growth) forms on the colon wall.

I

A benign, precancerous tumor grows.

Activation of oncogene ras.

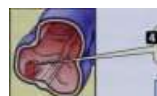
J



A class II adenoma (benign) grows.

I

Loss of tumor suppressor gene DCC



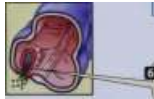
I

| A class III adenoma (benign) grows.

Loss of tumor suppressor gene p53



A carcinoma (malignant tumor) develops.



Other changes; loss of anti-metastasis gene

I

The cancer metastasizes (spreads to other tissues).

18.18 Multiple Mutations Transform a Normal Colon Epithelial Cell into a Cancerous Cell

In this form of cancer, at least five genes are mutated in a single cell.

place over many years of exposure to natural and human-made carcinogens and to spontaneous mutations.

The characterization of the molecular changes in tumor cells has opened up the possibility of genetic diagnosis and screening for cancer, as is done for genetic diseases. Many tumors are now commonly diagnosed in part by specific oligonucleotide probes for oncogene and/or tumor suppressor gene alterations. It is also possible to detect early in life whether an individual has inherited a mutated tumor sup-

pressor gene. For example, a person who inherits mutated copies of the tumor suppressor genes involved in colon cancer normally would have a high probability of developing this cancer by age 40. Surgical removal of the colon would prevent this metastatic tumor from arising.

Treating Genetic Diseases

Most treatments of genetic diseases try to alleviate the symptoms that affect the patient. But to effectively treat diseases caused by genes—whether they affect all cells, as in inherited disorders such as PKU, or only somatic cells, as in cancer—physicians must be able to diagnose the disease accurately, must know how the disease works at the biochemical level, and must be able to intervene early, before the disease ravages or kills the individual.

Basic research has provided the knowledge needed for accurate diagnostic tests, as well as a beginning at understanding pathogenesis (the cause of diseases) at the molecular level. Physicians are now applying this knowledge to treat genetic diseases. In this section, we will see that these treatments range from specifically modifying the mutant phenotype to supplying the normal version of a mutant gene.

One approach to treatment is to modify the phenotype

There are three ways to alter the phenotype of a genetic disease so that it no longer harms an individual.

restricting the substrate. Restricting the substrate of a deficient enzyme is the approach taken when a newborn is diagnosed with PKU. In this case, the deficient enzyme is phenylalanine hydroxylase, and the substrate is phenylalanine. The infant's inability to break down the phenylalanine in food leads to a buildup of the substrate, which causes the clinical symptoms. So the infant is immediately put on a special diet that contains only enough phenylalanine for immediate use.

Lofenelac, a milk-based product that is low in phenylalanine, is fed to these infants just like formula. Later, certain fruits, vegetables, cereals, and noodles low in phenylalanine can be added to the diet. Meat, fish, eggs, dairy products, and bread, which contain high amounts of phenylalanine, must be avoided, especially during childhood, when brain development is most rapid. The artificial sweetener aspartame must also be avoided, because it is made of two amino acids, one of which is phenylalanine.

People with PKU are generally advised to stay on a low-phenylalanine diet for life. Although maintaining these dietary restrictions may be difficult, it is effective. Numerous follow-up studies since newborn screening was initiated have shown that people with PKU who stay on the diet are no different from the rest of the population in terms of mental ability. This is an impressive achievement in public health, given the extent of mental retardation in untreated patients.

5-Fluorouracil blocks the synthesis of nucleotides.

Etoposide prevents DNA from unwinding, blocking DNA replication and transcription.

.

T

Arabinocytosine blocks DNA replication.

I\ DNA I

Transcription ^ --- j

T

Adriamycin blocks transcription to inhibit protein synthesis.

mRNA

Translation

Vincristine and Taxol block the mitotic spindle microtubules

.....T<>T* ^H from functioning.

■ * ■ ■ *

Cell division proteins

18.19 Drug Strategies for Killing Cancer Cells

The medications used against cancer attack rapidly dividing cancer cells in several ways. Unfortunately, most of them also affect noncancerous dividing cells.

metabolic inhibitors. As we described earlier, people with familial hypercholesterolemia accumulate dangerous levels of cholesterol in their blood. Not only are these people unable to metabolize dietary cholesterol, they also synthesize a lot of it. One effective treatment for people with this disease is the drug mevinolin, which blocks the patients' own cholesterol synthesis. Patients who receive this drug need only worry about cholesterol in their diet, and not about what their cells are making.

Metabolic inhibitors also form the basis of cancer therapy with drugs. The strategy is to kill rapidly dividing cells, since rapid cell division is the hallmark of malignancy. Many drugs kill dividing cells (Figure 18.19), but most of these drugs are given in the blood and thus also damage other, noncancerous, dividing cells in the body. Therefore, it is not surprising that people undergoing chemotherapy suffer side effects such as loss of hair (due to damage to the skin epithelium), digestive upsets (gut epithelial cells), and anemia (bone marrow stem cells). The effective dose of these highly toxic drugs for treating the cancer is often just below the dose that would kill the patient, so they must be used with utmost care. Often they can control the spread of cancer, but not cure it.

supplying the missing protein. An obvious way to treat a disease phenotype in which a functional protein is missing is to supply the missing protein. This approach is the basis of treatment of hemophilia A, in which the missing blood clotting factor is supplied in pure form. The production of human clotting protein by biotechnology has made it possible for a pure protein to be given instead of crude blood products, which could be contaminated with the AIDS virus or other pathogens.

MOLECULAR BIOLOGY AND MEDICINE 347

Unfortunately, the phenotypes of many diseases caused by genetic mutations are very complex. Simple interventions, such as some of those we have described, do not work for most such diseases. Indeed, a recent survey showed that current therapies for 351 diseases caused by single-gene mutations improved the patients' life span by only 15 percent.

Gene therapy offers the hope of specific treatments

Perhaps the most obvious thing to do when a cell lacks a functional allele is to provide one. Such gene therapy approaches are under intensive investigation for diseases ranging from the rare inherited disorders caused by single mutations, to cancer, AIDS, and atherosclerosis.

Gene therapy in humans seeks to insert a new gene that will be expressed in the host. Thus, the new DNA is often attached to a promoter that will be active in human cells. Presenting the DNA for cellular uptake follows the methods used in biotechnology: Calcium salts, liposomes, and viral vectors are used to get the "good gene" into the human cells. The physicians who are developing this "molecular medicine" are confronted by all the challenges of genetic engineering: effective vectors, efficient uptake, appropriate expression and processing of mRNA and protein, and selection within the body for the cells that contain the recombinant DNA.

Which human cells should be the targets of gene therapy? The best approach would be to replace the nonfunctional allele with a functional one in every cell of the body. But vectors to do this are simply not available, and delivery to every cell poses a formidable challenge. Until recently, the major attempts at gene therapy have been ex vivo. That is, physicians have taken cells from the patient's body, added the new gene to the cells in the laboratory, and then returned the cells to the patient in the hope that the correct gene product would be made (Figure 18.20).

A widely publicized example of this approach was the introduction of a functional gene for the enzyme adenosine deaminase into the white blood cells of a girl with a genetic deficiency of this enzyme. Unfortunately, these were mature white blood cells, and although they survived for a time in the girl, and provided some therapeutic benefit, they eventually died, as is the normal fate of such cells. It would be more effective to insert the functional gene into stem cells — the bone marrow cells that constantly divide to produce white blood cells. The use of stem cells is a major thrust of many current clinical experiments on gene therapy.

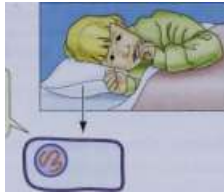
The other approach to gene therapy is to insert the gene directly into cells in the body of the patient. This in vivo approach is

being attempted for various types of cancer. For example, lung cancer cells are accessible if the DNA or vector is given as an aerosol through the respiratory system. Vectors carrying functional alleles of the tumor suppressor genes that are mutated in the tumors, as well as vectors expressing antisense RNA's against oncogene mRNA's, have been successfully introduced in this way to patients with lung cancer, with some clinical improvement.

348 CHAPTER EIGHTEEN

Sick patient

Q Isolated somatic cells from the patient are homozygous for the defective allele.



Somatic cell

IA copy of the normal allele is inserted into viral DNA.



I Isolated somatic cells are infected with the virus containing the recombinant DNA.

| The viral DNA carrying the normal allele inserts into the patient's somatic cell chromosome.

I Somatic cells containing the normal allele are cultured.

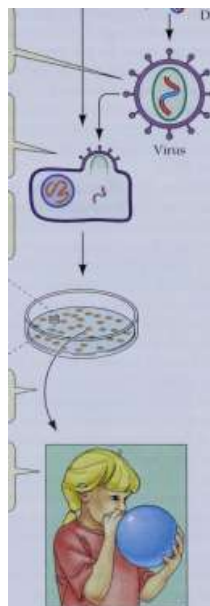
Viral DNA Normal allele

W^{^^}Jf Recombinant p DNA



Cultured cells are injected into the patient.

Q Symptoms are relieved by expression of the normal allele.



Well patient

Several thousand patients, over half of them with cancer, have undergone gene therapy. Most of these clinical trials have been at a preliminary level, in which people are given the therapy to see whether it has any toxicity, and whether the new gene is actually incorporated into the genome of the patient. More ambitious trials are under way, in which a

New genes are added to somatic cells taken from a patient's body, then returned to the body to make the missing gene product.

larger number of patients receive the therapy with the hope that their disease will disappear, or at least improve.

Sequencing the Human Genome

In 1984, the United States government sponsored a conference to examine the problem of detecting DNA damage in people exposed to low levels of radiation, such as those who had survived the atomic bomb in Japan 39 years earlier. Scientists attending this conference quickly realized that the ability to detect such damage would also be useful in evaluating environmental mutagens. But in order to detect changes in the human genome, scientists first needed to know its normal sequence.

In 1986, Renato Dulbecco, who won the Nobel prize for his pioneering work on cancer viruses, suggested that determining the sequence of human DNA could also be a boon to cancer research. He proposed that the scientific community be mobilized for the task. The result was the publicly funded Human Genome Project, an international effort. In the 1990s, private industry launched their own sequencing effort. By the summer of 2000, "draft" sequences of the human genome were ready. The final sequence is expected to be completed by 2003.

There are two approaches to genome sequencing

Each human chromosome consists of one double-stranded molecule of DNA. Because of their differing sizes, the 46 human chromosomes can be separated from each other and identified (see Figure 9.14). So it is possible to carefully isolate the DNA of each chromosome for sequencing. The straightforward approach would be to start at one end and simply sequence the entire 50 million base pairs of the chromosome. Unfortunately, this approach does not work.

The DNA of a molecule that is 50 million base pairs long cannot be sequenced all at once; only about 700 base pairs at a time can be sequenced. (See Figure 11.21 to review the DNA sequencing technique.) To sequence the entire genome, chromosomal DNA is first cut into fragments about 500 base pairs long, then each fragment is sequenced. For the human genome, which has about 3 billion base pairs, there are more than 6 million such fragments.

The problem then becomes putting these millions of pieces of the jigsaw puzzle back together. This problem can be overcome by breaking up the DNA in several ways into "sub-jigsaws" that overlap, and aligning the overlapping fragments. There are two ways to do this.

hierarchical sequencing. The publicly funded effort first systematically identified short marker sequences along the chromosomes, so that every small sequenced section of DNA would contain a marker. Then sequences with the common

RESEARCH METHOD

f

Hierarchical sequencing

A human chromosome is stained to reveal its bands

III Mill III ■ ■ ■ ■ ■ ITTl

n One 5 million bp band is removed and isolated for analysis.



D.\A L

i r

i r

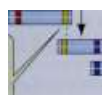
J L

"i r

J L

| The DNA is cut into large 55,000-2 million bp fragments by a restriction enzyme. The fragments are cloned in bacteria artificial chromosome (BAC) vectors.

| Sequence-tagged sites (STS) are identified on the fragments; common ones indicate overlaps.





o The BAC fragments are cut into small pieces and sequenced from STS to STS, 500 bp at a time.



markers could then be aligned (Figure 18.21, left panel). There are two types of mapped markers along the chromosome:

► Physical map markers are chromosomal landmarks that can be ordered and the distances between them determined. The result can be compared to a road map showing towns with the mileage separating them. The "towns" are DNA markers, and the "mileage" is in base pairs.

► Genetic map markers are specific DNA sequences, such as RFLPs or short simple sequence repeats, whose locations are determined genetically.

The simplest of the physical map markers are the recognition sites for restriction enzymes. Since there are hundreds of these recognition site-restriction enzyme pairs, there are hundreds of ways to cut the DNA and then generate a restriction map. Restriction mapping has been useful in generating maps for relatively small chromosome regions of thousands of base pairs.

Some restriction enzymes recognize 8-12 base pairs in DNA, not just the usual 4-6 base pairs. A DNA molecule with several million base pairs will have relatively few of these larger sites, and thus the enzyme will generate a small number of relatively large fragments. These large

Shotgun sequencing

Human DNA is randomly broken into 500-800 bp fragments

Each fragment is sequenced.



i r

J L

"i r

i r

J L

J L

i r

J L

"i r

J L

i nr

i r

J L

i r

J L

i r

i r

i r

i r
J L
J L
"i r
J L
i i i r i r
i i i u L
|Jac

A computer finds overlapping sequences shared by a fragment, and the fragments are aligned.



[Tfim. 18-2 1 Two Approaches to Sequencing DNA

In the hierarchical approach (left), markers are mapped and the DNA fragments are then aligned by matching overlapping marked sites whose sequence is known (sequence-tagged sites, STS). In the shotgun approach (right), the DNA is fragmented and a computer is used to find the markers.

fragments can be put into a vector called a bacterial artificial chromosome (BAC), which can carry about 250,000 base pairs of inserted DNA in a single copy and cloned in bacteria to create a DNA library.

The books (fragments) in this library can be arranged in the proper order by using sequence-tagged sites (STS's). These are unique stretches of DNA, 60 to 100 base pairs long, whose sequences are known. About 41,000 STS's have been precisely mapped on human chromosomes, meaning that each is less than 100,000 base pairs (or just a few genes) away from the next STS.

To arrange DNA fragments on a map, libraries made from different restriction enzyme cuts are compared. If two large fragments of DNA cut with different enzymes have the same STS, they must overlap.

Genetic map markers are also useful tools in analyzing the genome. As we described earlier, linkage studies with markers have been very important in tracking down disease-causing genes by positional cloning. The genetic and physical map markers can complement each other— one providing new markers for the other.

shotgun sequencing. Instead of the "top-down" approach—getting map makers, then fragmenting the DNA and sequencing it—the "shotgun" approach cuts the DNA at random into small, sequence-ready fragments and lets

350 CHAPTER EIGHTEEN

18.22 Is This the Future of Medicine?

The elucidation of the human genome sequence may result in an approach to medicine that is oriented to the genetic and functional individuality of each patient.

Diagnostics

Disease with genetic component

powerful computers determine markers that overlap (Figure 18.21, right panel). The fragments can then be aligned.

The shotgun method, which has been used by private industry, is much faster than the hierarchical approach because there is no need for physical or genetic maps. At first there was considerable skepticism about the method. There were concerns that without rigorous prior mapping of marker sites on the chromosomes, the computer might pick out repetitive sequences common to many DNA fragments and line the fragments up incorrectly. But the rapid rate of development of sophisticated computer and software power has allowed the technique to quickly be refined to a point where inaccurate alignment is not a major problem.

The entire 180 million-base-pair fruit fly genome (see Chapter 14) was sequenced by the shotgun method in little over a year. This proved that the rapid method might work for the much larger human genome, and in fact it did.

The human genome is more than just a sequence

Reading the human "book of life" is an achievement that ranks with other recent great events in exploration, such as landing on the moon. But gene sequencing and the tools developed to carry it out, are changing biology in many other ways as well:

► The sequences of other organisms have provided insights and practical information on both prokaryotic (Chapter 13) and eukaryotic (Chapter 14) genomes. Many genes sequenced and identified in "simpler" organisms have counterparts in humans, so these findings have facilitated the identification of human genes.

► Mapping technology has made the isolation of human genes by positional cloning much easier, because of the many chromosome markers available. Over 100 disease-related genes have been identified in this way.

► Genetic variability in drug metabolism has been a medical problem for a long time. The identification of the genes responsible is leading to tests that predict who will react best to which medications. This is the emerging science of pharmacogenomics.

► DNA chips (see Figure 17.12) are being used to analyze the specific expression of thousands of genes in cells in different biochemical states. For example, a Cancer Genome Anatomy Project seeks to make an mRNA "fingerprint" of a tumor at each stage of its development. Finding out which sequences are expressed at what stage will be important not only in diagnosis, but also to identify targets for gene therapy.

► The Human Genome Diversity Project is looking for important polymorphisms in specific human popula-

s*

Pharmacogenomics

Preventive medicine

/

Map gene

Clone gene

Gene therapy

\

Understand basic biological defect

Drug therapy



Time

tions. For example, the Pima Indians in Arizona have a high frequency of extreme obesity and diabetes. A search of their genomes might reveal genes predisposing them to these diseases.

The end result of all of this knowledge of the human genome may lead to a new approach to medical care, in which each person's genes will be used to prescribe lifestyles and treatments that can maximize that person's genetic potential (Figure 18.22).

How should genetic information be used?

After the primary genetic defect that causes cystic fibrosis was discovered, many people predicted a "tidal wave" of genetic testing for heterozygous carriers. Everyone would want the test, it was thought—especially the relatives of patients with the genetic disease. But the tidal wave has not developed. To find out why, a team of psychologists, ethicists, and geneticists interviewed 20,000 people in the United States. What the researchers found surprised them: Most people are simply not very interested in their genetic makeup, unless they have a close relative with a genetic disease and are involved in a decision about pregnancy.

There are other people, however, who might be very interested in the results of genetic testing. People who test positive for genetic abnormalities, from hypercholesterolemia to cancer, might be denied employment or health insurance. The linking of genetic abnormalities to behavioral characteristics, such as manic depression and schizophrenia, has led to the potential for screening and then social manipulations of those at risk. Consequently, many legislative bodies are considering and passing bills that prohibit genetic discrimination.

The Human Genome Diversity Project has raised many concerns about exploitation and commercialization of people's DNA sequences. Is a gene that confers resistance to cancer, for example, the property of an individual, an ethnic group in which it may be frequent, a pharmaceutical company that finds it, or humanity at large? This issue of ownership is being tested worldwide, perhaps no more acutely than in Iceland, most of whose 270,000 people trace their ancestry back to the first settlers that arrived on the island

MOLECULAR BIOLOGY AND MEDICINE 351

1,000 years ago. Tissues from the entire population have been sampled and stored for several generations. This tissue bank is a potential gold mine for genetic prospectors, and a single company has been set up, with government support, to sell the knowledge that comes out of analyzing the DNA's of Iceland's people.

n



Chapter Summary

Protein as Phenotype

- ▶ In many human genetic diseases, a single protein is missing or nonfunctional. Therefore, the one-gene, one-poly peptide relationship applies to human genetic diseases. Review Figure 18.1
- ▶ A mutation in a single gene causes alterations in its protein product that may lead to clinical abnormalities or have no effect. Review Figure 18.2
- ▶ Some diseases are caused by mutations that affect structural proteins; examples include Duchenne's muscular dystrophy and hemophilia.
- ▶ The genes that code for receptors and membrane transport proteins can also be mutated and cause diseases such as familial hypercholesterolemia and cystic fibrosis. Review Figure 18.3
- ▶ Prion diseases are caused by a protein with an altered shape that is transmitted from one person to another and alters the same protein in the second person. Review Figure 18.4
- ▶ Few human diseases are caused by a single-gene mutation. Most are caused by the interactions of many genes and proteins with the environment.
- ▶ Human genetic diseases show different patterns of inheritance. Mutant alleles may be inherited as autosomal recessives, autosomal dominants, X-linked conditions, or chromosomal abnormalities.

Mutations and Human Diseases

- ▶ Molecular biology techniques have made possible the isolation of many genes responsible for human diseases.
- ▶ One method of identifying the gene responsible for a disease is to isolate the mRNA for the protein in question and then use the mRNA to isolate the gene from a gene library. DNA from a patient that lacks a piece of a chromosome can be compared with DNA from a person who does not show this deletion to isolate a missing gene. Review Figure 18.6
- ▶ In positional cloning, DNA markers are used as guides to point the way to a gene. These markers may be restriction fragment length polymorphisms that are linked to a mutant gene. Review Figure 18.7
- ▶ Human mutations range from single point mutations to large deletions. Some of the most common mutations occur where the modified base 5-methylcytosine is converted to thymine. Review Figure 18.8
- ▶ The effects of the fragile-X chromosome worsen with each generation. This pattern is caused by a triplet repeat that tends to expand with each new generation. Review Figure 18.9
- ▶ Genomic imprinting results in a gene being differentially expressed depending on which parent it comes from.

Detecting Human Genetic Variations

- ▶ Genetic screening detects human gene mutations. Some protein abnormalities can be detected by simple tests, such as tests for the presence of excess substrate or lack of product. Review Figure 18.10
- ▶ The advantage of testing DNA for mutations directly is that any cell can be tested at any time in the life cycle.
- ▶ There are two methods of DNA testing: allele-specific cleavage and allele-specific oligonucleotide hybridization. Review Figures 18.11, 18.12

Cancer: A Disease of Genetic Changes

- ▶ Tumors may be benign, growing only to a certain extent and then stopping, or malignant, spreading through organs and to other parts of the body.
- ▶ At least five types of human cancers are caused by viruses, which account for about 15 percent of all cancers. Review Table 18.2
- ▶ Eighty-five percent of human cancers are caused by genetic mutations of somatic cells. These mutations occur most commonly in dividing cells. Review Figure 18.14
- ▶ Normal cells contain proto-oncogenes, which, when mutated, can become activated and cause cancer by stimulating cell division or preventing cell death. Review Figure 18.15
- ▶ About 10 percent of all cancer is inherited as a result of the mutation of tumor suppressor genes, which normally act to slow down the cell cycle. For cancer to develop, both alleles of a tumor suppressor gene must be mutated.
- ▶ In inherited cancer, an individual inherits one mutant allele and then somatic mutation occurs in the second one. In

sporadic cancer, two normal alleles are inherited, so two mutational events must occur in the same somatic cell. Review Figures 18.16, 18.17

► Mutations must activate several oncogenes and inactivate several tumor suppressor genes for a cell to produce a malignant tumor. Review Figure 18.18

Treating Genetic Diseases

► Most genetic diseases are treated symptomatically. However, as more knowledge is accumulated, specific treatments are being devised.

► One treatment approach is to modify the phenotype—for example, by manipulating the diet, providing specific metabolic inhibitors to prevent the accumulation of a harmful substrate, or supplying a missing protein. Review Figure 18.19

► In gene therapy, a mutant gene is replaced with a normal gene. Either the affected cells can be removed, the new gene added, and the cells returned to the body, or the new gene can be inserted via a vector directly into the patient. Review Figure 18.20

Sequencing the Human Genome

► Human genome sequencing is determining the entire human DNA sequence, which requires sequencing many 500-base-pair fragments and then fitting the sequences back together.

► In hierarchical gene sequencing, marker sequences are identified and mapped. The markers are then sought in sequenced fragments and are used to align the fragments. In the shotgun approach, the fragments are sequenced and then common markers are identified by computer. Review Figure 18.21

► The identification of more than 30,000 human genes may lead to a new molecular medicine. Review Figure 18.22

► As more genes relevant to human health are described, concerns about how such information is used are growing.

352 CHAPTER EIGHTEEN

For Discussion

1. Compare the roles of proto-oncogenes and tumor suppressor genes in normal cells. How do these genes and their functions change in tumor cells? Propose targets for cancer therapy involving these gene products.
2. In the past, it was common for people with phenylketonuria (PKU) who were placed on a low-phenylalanine diet after birth to be allowed to return to a normal diet during their teenage years. Although the levels of phenylalanine in their blood were high, their brains were thought to be beyond the stage of being harmed. If a woman with PKU becomes pregnant, however, a problem arises. Typically, the fetus is heterozygous, but is unable at early stages of development to metabolize the high levels of phenylalanine that arrive from the mother's blood. What is the fetus heterozygous? What do you think would happen to the fetus during this "maternal PKU" situation? What would be your advice to a woman with PKU who wants to have a child?
3. A "knockout" mouse has been made that has deletions in both copies of the gene coding for PrP^{sc}. Would you expect this mouse to develop a prion disease if infected with PrP^{sc}? Explain your answer.
4. Cystic fibrosis is an autosomal recessive disease in which thick mucus is produced in the lungs and airway. The gene responsible for this disease codes for a protein composed of 1,480 amino acids. In most patients with cystic fibrosis, the protein has 1,479 amino acids: A phenylalanine is missing at position 508. How would you test the older brother of a baby with cystic fibrosis to determine whether he is a carrier for the disease? How would you design a gene therapy protocol to "cure" the cells in the lung and airway?



Natural Defenses against Disease



On January 6, 1777, George Washington, commanding the Revolutionary army of the fledgling United States, made a fateful medical/military decision. As he wrote to his chief physician, "Finding smallpox to be spreading much, and fearing that no precaution can prevent it from running through the whole of our army, I have determined that the troops shall be inoculated. Should the disease rage with its usual virulence, we should have more to dread from it than the sword of the enemy."

Washington was speaking from experience. He himself had survived the disease in 1751. During 1776 his army lost

l^OOjneriiniiaile^^ This hi g hl y vir-

ulent disease, which killed up to 1 in 4 people who were exposed to it, had already figured prominently in American history. A century before, it had decimated the Native Americans, making colonization by Europeans easier. Two years previously at Quebec, it had laid waste to an American army that was trying to annex Canada by force.

The death rate due to smallpox in the Revolutionary army plummeted after Washington's order was carried out. How did inoculation, a practice that was learned from the people in the Near East and from African slaves, save the soldiers? And why was Washington himself immune to the disease as it ravaged his army?

The answers to these questions lie in the cells and molecules of the immune system. When Washington caught smallpox as a teenager, cells called macrophages engulfed some of the smallpox viruses by phagocytosis and partly digested them. The macrophages displayed fragments of the viruses on their cell surfaces. Specialized white blood cells called T cells recognized those fragments and were activated to divide and differentiate further. The descendants of these activated T cells then attacked Washington's virus-infected cells, preventing the lethal spread of the disease. Other descendants of the T cells persisted in his body as "memory cells" and rapidly defended him when he was exposed to the disease as an adult. Inoculation of his soldiers with powdered scabs containing dead viruses from smallpox patients stimulated the formation of these memory cells in their bodies, preventing the virus from spreading following infection. This practice had been

George Washington

Washington's decision to immunize his army against smallpox saved many lives and probably helped him win the Revolutionary War.

used for centuries, and was finally put on a more scientific basis by Edward Jenner two decades after Washington's army was inoculated.

These defensive events in the bodies of Washington and his soldiers required the participation of many kinds of proteins. Some cellular proteins functioned as specific receptors, such as the markers identifying Washington's cells, some as signals triggering events in the macrophages and T cells, and others as weapons leading to the breakdown of infected cells.

Animal defense systems are based on the distinction between self—the animal's own molecules—and nonself, or foreign, molecules. In this chapter we consider the mechanisms by which animals recognize nonself molecules and combat infection and disease. Many of these mechanisms are based on the principles of genetics and molecular biology that have been discussed in earlier chapters.

In general, there are two types of defenses. Innate, or nonspecific, defenses are general mechanisms that protect the body from many pathogens. An example is the skin, which acts as a barrier to stop potentially invading viruses



354 CHAPTER NINETEEN

from entering the body. Most animals and plants have innate defenses. Specific defenses are aimed at a single target. For example, an antibody protein can be made that binds to a virus if that virus ever enters the bloodstream, and this binding results in the virus being destroyed. Specific defense mechanisms are present in vertebrate animals. In animals that have both, innate and specific defenses operate together and offer a coordinated defense.

Defensive Cells and Proteins

The components of the defense system are dispersed throughout the body and interact with almost all of its other tissues and organs. The lymphoid tissues, which include the thymus, bone marrow, spleen, and lymph nodes, are essential parts of our defense system (Figure 19.1), but central to their functioning are the blood and lymph.

Blood and lymph are fluid tissues that consist of water, dissolved solutes, and cells. Blood plasma is a yellowish solution containing ions, small molecular solutes, and soluble proteins. Suspended in the plasma are red blood cells, white blood cells, and platelets (cell fragments essential to

In the lymph nodes, fluids are filtered and white blood cells mature.

Lymph ducts conduct lymph.

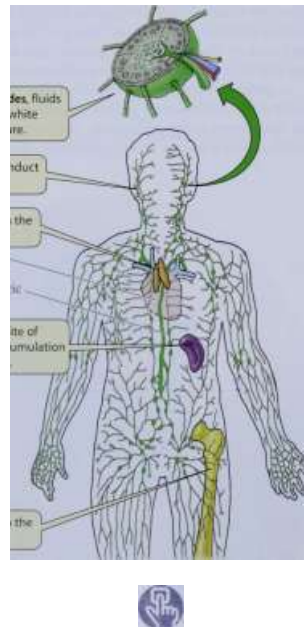
T cells mature in the thymus.

Heart

Thoracic duct

The spleen is a site of lymphocyte accumulation and maturation.

B cells mature in the bone marrow.



19.1 The Human Defense System

A network of ducts collects lymph from the body's tissues and carries it toward the heart, where it mixes with blood to be pumped back to the tissues. The thymus, spleen, and bone marrow are essential to the body's defensive network.

clotting) (Figure 19.2). While red blood cells are normally confined to the closed circulatory system of (the heart, arteries, capillaries, and veins), white blood cells and platelets are also found in the lymph. Lymph is a fluid derived from blood and other tissues. It accumulates in spaces outside the blood vessels and contains many of the components of blood, but not red blood cells. From the spaces around body cells, the lymph moves slowly into the lymphatic system. Tiny lymph capillaries conduct fluid to larger vessels that join together, forming one large lymph duct that joins a major vein (the left subclavian vein) near the heart. By this system of vessels, the lymphatic fluid is eventually returned to the blood and the circulatory system.

At many sites along the lymph vessels are small, roundish structures called lymph nodes, which contain a variety of white blood cells. As fluid passes through the node, it is filtered and "inspected" for foreign materials by these defensive cells.

White blood cells play many defensive roles

One milliliter of blood typically contains about 5 billion red blood cells and 7 million of the larger white blood cells. White blood cells have nuclei and are colorless, whereas mammalian red blood cells lose their nuclei during development. White blood cells can leave the circulatory system and enter extracellular spaces where foreign cells or substances are present. In response to invading pathogens, the number of white blood cells in the blood and lymph may rise sharply. Providing medical professionals with a useful clue for detecting an infection.

Several types of white blood cells are important in the body's defenses. But they are all members of two broad groups, phagocytes and lymphocytes. Phagocytes engulf and digest nonself materials. The most important phagocytes are the neutrophils and the macrophages. In addition to phagocytosis of nonself materials, macrophages have the important additional function of presenting partly digested nonself materials to the T cells.

Lymphocytes are the most abundant white cells. A healthy person has about a trillion lymphocytes, making them as numerous as brain cells. There are two types of lymphocytes: B cells and T cells. Both originate from cells in the bone marrow. Immature T cells migrate via the blood to the thymus, where they mature. They participate in specific defenses against foreign or altered cells, such as virus-infected cells and tumor cells. The B cells leave the bone marrow and circulate through the blood and lymph vessels. B cells make specialized proteins called antibodies that enter the blood and bind to nonself substances.

Immune system proteins bind pathogens or signal other cells

The cells that defend our bodies work together like cast members in a drama, interacting with one another and with the cells of invading pathogens. These cell-cell interactions are accomplished by a variety of key proteins, including re-

NATURAL DEFENSES AGAINST DISEASE 355

TYPE OF CELL

FUNCTION

Myeloid

progenitor

cell

Red blood cells

(erythrocytes)

Platelets

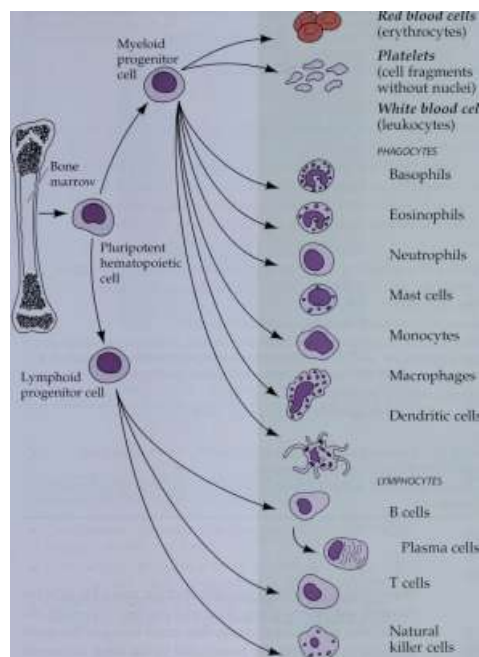
(cell fragments without nuclei)

White blood cells

(leukocytes)

PHAGOCYTES

Basophils Eosinophils Neutrophils Mast cells



Transport oxygen and carbon dioxide Initiate blood clotting

Lymphoid progenitor cell

Monocytes Macrophages Dendritic cells

Release histamine; may promote the development of T cells

Kill antibody-coated parasites Phagocytize antibody-coated pathogens Release histamine when they are damaged Develop into macrophages

Engulf and digest microorganisms; activate T cells

Present antigens to T cells

Natural killer cells

Differentiate to form antibody-producing cells

and memory cells Secrete antibodies

Kill virus-infected cells; regulate activities of other white blood cells

Attack and lyse virus-infected or cancerous body cells



79.2 Blood Cells

Pluripotent stem cells in the bone marrow can differentiate into red blood cells, platelets, and the various types of white blood cells.

ceptors, surface markers, signaling molecules, and toxins. They will be discussed later in the chapter, as they appear in the context of our story. However, let's take a brief look at four of the major players here.

▶ **Antibodies** are proteins that bind specifically to certain substances identified by the immune system as nonself or altered self. B cells secrete antibodies as defensive

~gaps.

▶ **T receptors** are proteins on the surfaces of T cells. They recognize and bind to nonself substances on the surfaces of other cells.

▶ **Major histocompatibility complex (MHC)** proteins protrude from the surfaces of most cells in the mammalian body. They are important "self-identifying

labels and play major parts in coordinating interactions among T cells and macrophages. ▶ **Cytokines** are soluble signal proteins released by T cells, macrophages, and other cells. They bind to and alter the behavior of their target cells. Different cytokines activate or inactivate B cells, macrophages, and T cells. Some cytokines limit tumor growth by killing tumor cells.

Innate Defenses

Innate defenses are general protection mechanisms to stop pathogens —harmful organisms that can cause disease— from invading the body. In humans, these defenses include physical barriers as well as cellular and chemical defenses. Table 19.1 provides a summary of the innate defense mechanisms.

356 CHAPTER NINETEEN

19.1

Human Innate Defenses

DEFENSIVE AGENT

FUNCTION

Surface barriers

Skin

Acid secretions

Mucus membranes

Mucus secretions

Nasal hairs

Cilia

Gastric juice

Acid in vagina

Tears, saliva

Nonspecific cellular, chemical,

Normal flora

Fever

Coughing, sneezing Inflammatory response (involves blood plasma and phagocyte

Phagocytes (macrophages and neutrophils) Natural killer cells Antimicrobial proteins Interferon

Complement proteins

Prevents entry of pathogens and foreign substances

Inhibit bacterial growth on skin

Prevent entry of pathogens

Trap bacteria and other pathogens in digestive and respiratory tracts

Filter bacteria in nasal passages

Move mucus and trapped materials away from respiratory passages

Concentrated HCl and proteases destroy pathogens in stomach

Limits growth of fungi and bacteria in female reproductive tract

Lubricate and cleanse; contain lysozyme, which destroys bacteria

and coordinated defenses

Compete with pathogens; may produce substances toxic to pathogens Body-wide response inhibits microbial multiplication and speeds body

repair processes Expels pathogens from upper respiratory passages

Limits spread of pathogens to neighboring tissues; concentrates defenses; digests pathogens and dead tissue cells; released chemical mediators attract phagocytes and specific defense lymphocytes to site Engulf and destroy pathogens that enter body Attack and lyse virus-infected or cancerous body cells

es leakage of

s from capillaries)

Released by virus-infected cells to protect healthy tissue from viral

infection; mobilizes specific defenses Lyse microorganisms, enhance phagocytosis, and assist in inflammatory response

Barriers and local agents defend the body against invaders

Skin is a primary innate defense against invasion. Fungi, bacteria, and viruses rarely penetrate healthy, unbroken skin. But damaged skin or internal surface tissue greatly increase the risk of infection by pathogenic agents.

The bacteria and fungi that normally live and reproduce in great numbers on our body surfaces without causing disease are referred to as normal flora. These natural occupants of our bodies compete with pathogens for space and nutrients, so normal flora are a form of innate defense.

The mucus-secreting tissues found in parts of the visual, respiratory, digestive, excretory, and reproductive systems have other defenses against pathogens. Tears, nasal mucus, and saliva possess an enzyme called lysozyme that attacks the cell walls of many bacteria. Mucus in the nose traps airborne microorganisms, and most of those that get past this filter end up trapped in mucus deeper in the respiratory tract. Mucus and trapped pathogens are removed by the beating of cilia in the respiratory passageway, which moves a sheet of mucus and the debris it contains up toward the nose and mouth. Sneezing is another way to remove microorganisms from the respiratory tract.

Pathogens that reach the digestive tract (stomach, small intestine, and large intestine) are met by other defenses.

The stomach is a deadly environment- for many bacteria because of the hydrochloric acid and protein-digesting enzymes that are secreted into it. The intact lining of the small intestine is not normally penetrated by bacteria, and some pathogens are killed by bile salts secreted into this part of the tract. The large intestine harbors many bacteria, which multiply freely; however, these are usually removed quickly with the feces. Most of the bacteria in the large intestine are normal flora that provide benefits to their host.

All of these barriers and secretions are nonspecific defenses because they act on all invading pathogens in the same way. But there are more complicated nonspecific cellular chemical defenses.

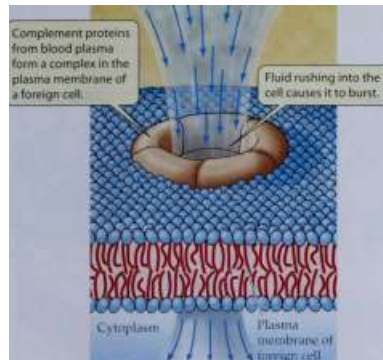
Innate defenses include chemical and cellular processes

Pathogens that manage to penetrate the body's outer and inner surfaces encounter additional nonspecific defenses. These defenses include the secretion of various defensive proteins as well as cellular defenses involving phagocytosis.

complement proteins. Vertebrate blood contains about 20 different antimicrobial proteins that make up the complement system. These proteins, in different combinations, provide three types of defenses. In each type, the comple-

Inrushing fluids

Complement proteins from blood plasma form a complex in the plasma membrane of a foreign cell.



Plasma membrane of foreign cell

7 9.3 Complement Proteins Destroy a Foreign Cell

Some complement proteins attach to foreign cells such as bacteria after antibodies have bound to them. The porelike structure of the protein allows fluids to pour into the foreign cell until it bursts.

ment proteins act in a characteristic sequence, or cascade, with each protein activating the next.

- Complement proteins attach to microbes, which helps phagocytes destroy them:
- Complement proteins activate the inflammatory response and attract phagocytes to sites of infection.
- Complement proteins, acting with antibodies, lyse (burst) invading cells such as bacteria (Figure 19.3).

interferons. When cells are infected by a virus, they produce small amounts of antimicrobial proteins called interferons that increase the resistance of neighboring cells to infection by the same or other viruses. Interferons have been found in many vertebrates and are one of the body's lines of nonspecific defense against viral infection.

Interferons differ from species to species, and each vertebrate species produces at least three different interferons. All interferons are glycoproteins (proteins with a carbohydrate component) consisting of about 160 amino acids. By binding to receptors in the plasma membranes of their target cells, interferons inhibit viral replication.

PHAGOCYTOSIS AND OTHER CELLULAR ASSAULTS. Phagocytes

provide an important nonspecific defense against pathogens that penetrate the surface of the host. Some phagocytes travel freely in the circulatory system; others can move out of blood vessels and adhere to certain tissues. Pathogens such as large molecules, cells, and viruses become attached to the membrane of a phagocyte (Figure 19.4), which ingests them by endocytosis. After lysosomes fuse with the phagosome,

NATURAL DEFENSES AGAINST DISEASE 357

the pathogens are degraded by lysosomal enzymes (see Figure 4.15).

There are three types of phagocytes:

- Neutrophils are the most abundant phagocytes, but they are relatively short-lived. They recognize and attack pathogens in infected tissue.
- Monocytes mature into macrophages, which live longer than neutrophils and can consume large numbers of pathogens. Some macrophages roam through the body; others reside permanently in lymph nodes, the spleen, and certain other lymphoid tissues, "inspecting" the lymph for pathogens.
- Eosinophils are weakly phagocytic. Their primary function is to kill parasites, such as worms, that have been coated by antibodies.

A class of nonphagocytic white blood cells, known as natural killer cells, can distinguish virus-infected cells and some tumor cells from their normal counterparts and initiate the lysis of these target cells. In addition to this nonspecific action, natural killer cells are part of the specific defenses, as we will describe later in this chapter.

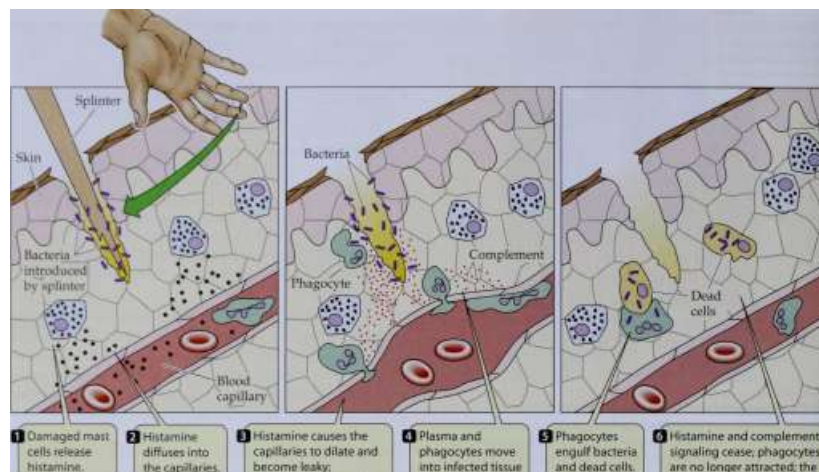
inflammation. The body employs the inflammation response in dealing with infection or with any other process that causes tissue injury either on the surface of the body or internally. You have experienced the symptoms of inflammation: redness and swelling, accompanied by heat and pain. The damaged body cells cause the inflammation by releasing various substances, such as the chemical signal histamine. Cells adhering to the skin and linings of organs, called mast cells, release histamine when they are damaged, as do white blood cells called basophils.



19.4 A Phagocyte and Its Bacterial Prey

Some bacteria (appearing yellow in this artificially colored scanning electron micrograph) have become attached to the surface of a phagocyte in the human bloodstream. Many of these bacteria will be engulfed by the phagocyte and destroyed before they can multiply and damage the human host. A single phagocyte can digest about 20 bacteria.

358 CHAPTER NINETEEN



Damaged mast cells release histamine.

I Histamine diffuses into the capillaries.

§J Histamine causes the capillaries to dilate and become leaky; complement proteins attract phagocytes.

Q Plasma and

phagocytes move into infected tissue from the capillary.

o Phagocytes engulf bacteria and dead cells.

o Histamine and complement signaling cease; phagocytes are no longer attracted; the tissue returns to normal.



7 9.5 Interactions of Cells and Chemical Signals in Inflammation

The histamine-induced swelling of the inflammation reaction is accompanied by redness, heat, and pain. The chemical signals associated with the reaction attract the phagocytes that are largely responsible for healing the wound.

The redness and heat of inflammation result from histamine-induced dilation of blood vessels in the infected or injured area (Figure 19.5). Histamine also causes the capillaries (the smallest blood vessels) to become leaky, allowing blood plasma and phagocytes to escape into the tissue, causing the characteristic swelling. The pain of inflammation results from increased pressure (from the swelling) and from the action of leaked enzymes.

In damaged or infected tissue, complement proteins and other chemical signals attract phagocytes—neutrophils first, and then monocytes, which become macrophages. The macrophages engulf the invaders and debris and are responsible for most of the healing associated with inflammation. They produce several cytokines, which, among other functions, signal the brain to produce a fever, which inhibits the growth of the invading pathogen. Cytokines may also attract phagocytic cells to the site of injury and initiate a specific immune response to the pathogen.

Following inflammation, pus accumulates. Pus is composed of dead cells (neutrophils and the damaged body cells) and leaked fluid. A normal result of inflammation, pus is gradually consumed and digested by macrophages.

Specific Defenses: The Immune Response

Nonspecific defenses are numerous and effective, but some invaders elude them and must be dealt with by defenses targeted against specific threats. The recognition and destruction of specific nonself substances is an important function of an animal's immune system.

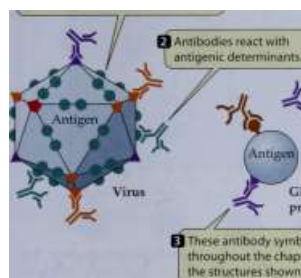
Four features characterize the immune response

The characteristic features of the immune system are specificity, the ability to respond to an enormous diversity of foreign molecules and organisms, the ability to distinguish self from nonself, and immunological memory.

specificity. Antigens are organisms or molecules that are recognized by and/or interact with the immune system to initiate an immune response. The specific sites on antigens that the immune system recognizes are called antigenic determinants (Figure 19.6). Chemically, an antigenic determinant is a specific portion of a large molecule, such as a sequence of amino acids that may be present in several proteins. A large antigen, such as a whole cell, may have many different antigenic determinants on its surface, each capable of being bound by a specific antibody or T cell. Even a single protein has multiple, different antigenic determinants. The host responds to the presence of an antigen by producing highly specific defenses—T cells or antibodies that correspond to the antigenic determinants of the antigen. Each T cell and each antibody is specific for a single antigenic determinant.

Q Antigenic determinants are a small part of antigens— for example, a part of a protein on a viral coat.

Antibodies react with antigenic determinants.



of Globular

^- proteins

These antibody symbols, used throughout the chapter, are based on the structures shown in Figure 19.11.

s

7 9.6 Each Antibody Matches an Antigenic Determinant

Each antigen has many different antigenic determinants that are recognized by specific antibodies. An antibody recognizes and binds to its antigenic determinant to initiate defensive measures against the antigen.

DIVERSITY. Challenges to the immune system are legion: individual molecules, viruses, bacteria, protists, and multicellular parasites. Each of these types of potential pathogens includes many species; each species includes many subtly differing genetic strains; each strain possesses multiple surface features, each of which is presented to the immune system. Estimates vary, but a reasonable guess is that humans can respond specifically to 10 million different antigenic determinants. Upon recognition of an antigenic determinant, the immune system responds by activating lymphocytes of the appropriate specificity.

distinguishing self from nonself. The human body contains tens of thousands of different proteins, each with a specific three-dimensional structure capable of generating an immune response. Every cell in the body bears a tremendous number of antigenic determinants. A crucial attribute of an individual's immune system is that it recognizes the body's own antigenic determinants and does not attack them. Failure to make this distinction may lead to an autoimmune disease—an attack on one's own body. Such diseases include rheumatoid arthritis and lupus.

immunological memory. After responding to a particular type of pathogen once, the immune system "remembers" that pathogen and can usually respond more rapidly and powerfully to the same threat in the future. This immunological memory usually saves us from repeats of childhood diseases such as chicken pox. Vaccination and inoculation against disease work because the immune system "remembers" the antigenic determinants that are inoculated into the body. —

There are two interactive immune responses

The immune system has two responses against invaders: the humoral immune response and the cellular immune response. The two responses operate in concert—simultaneously and cooperatively, sharing mechanisms.

In the humoral immune response (from the Latin humor, "fluid"), antibodies react with antigenic determinants on foreign invaders in blood, lymph, and tissue fluids. An animal produces a vast diversity of antibodies that, among them, can react with almost any conceivable antigen encountered. Some antibodies are soluble and travel free in the blood and lymph; others exist as integral membrane proteins on specialized lymphocytes called B cells.

The first time a specific antigen invades the body, it may be detected by and bind to a cell whose membrane antibody recognizes one of its antigenic determinants. This activated B cell forms a plasma cell that makes multiple soluble copies of antibody with the same specificity as the membrane antibody.

The cellular immune response is directed against an antigen that has become established within a cell of the host animal. It detects and destroys virus-infected or mutated cells.

Unlike the humoral response, the cellular immune response does not use antibodies. Instead, it is carried out by T cells within the lymph nodes, the bloodstream, and the interstitial spaces. The T cells have integral membrane proteins called T cell receptors—surface glycoproteins that recognize and bind to antigenic determinants while remaining part of the cell's plasma membrane. Like antibodies, T cell receptors have specific molecular configurations that bind to specific antigenic determinants. Once a T cell is bound to a determinant, it initiates an immune response.

Clonal selection accounts for the characteristic features of the immune response

Each person possesses an enormous number of different B cells and T cells, apparently capable of dealing with almost any antigen ever likely to be encountered. How does this diversity arise? How do B and T cells specific for certain antigens proliferate? And why don't our antibodies and T cells attack and destroy our own bodies? The versatility of immune responses, the proliferation of specific cells, the ability to distinguish between self and nonself, and immunological memory can all be explained by the theory of clonal selection.

According to clonal selection theory, each individual human contains an enormous variety of different B cells, and each type of B cell is able to produce only one kind of antibody. Thus there are millions of different B cells, each one producing a particular antibody and displaying it on the cell surface. When an antigen that fits this surface antibody binds to it, the B cell is activated. It divides to form a clone of cells, all of them producing that particular antibody. Thus, the selection of a particular antibody-producing B cell for proliferation (Figure 19.7). In the same way, an antigenic cell selects a T cell expressing a particular T cell receptor on its surface for proliferation.

360 CHAPTER NINETEEN

The clonal selection theory accounts nicely for the body's ability to respond rapidly to any of a vast number of different antigens. In the extreme case, even a single B cell might be sufficient for an immunological response by the body provided that it encounters its antigen and then proliferates into a large clone rapidly enough to combat the invasion.

Immunological memory and immunity result from clonal selection

According to clonal selection theory, an activated lymphocyte (B cell or T cell) produces two types of daughter cells, effector cells and memory cells.

► Effector cells carry out the attack on the antigen. They are effector cells that produce antibodies, or

T cells that, upon binding an antigenic determinant, release messenger molecules called cytokines. Effector cells live only a few days.

► Memory cells are long-lived cells that retain the ability to start dividing on short notice to produce more effector and more memory cells. Memory B and possibly

T cells may survive for decades.

When the body first encounters a particular antigen, a primary immune response is activated, and lymphocytes produce clones of effector and memory cells. The effector cells destroy the invaders at hand and then die, but one or more clones of memory cells have now been added to the immune system and provide immunological memory.

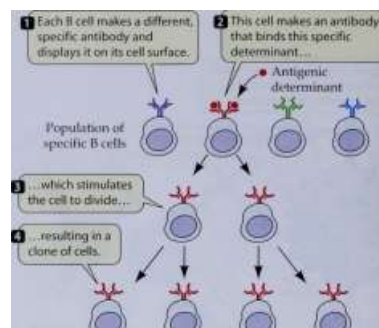
After the body's first immune response to a particular antigen, subsequent encounters with the same antigen will result in a greater and more rapid production of antigen-specific antibody or T cells. This response is called the secondary immune response. The first time a vertebrate animal is exposed to a particular antigen, there is a time lag (usually several days) before the number of antibody molecules and T cells slowly increases (Figure 19.8). But for years afterward—sometimes for life—the immune system "remembers" that particular antigen. The secondary immune response has a shorter lag time, a greater rate of antibody production, and a larger production of total antibody or T cells than the primary response.

Thanks to immunological memory, recovery from many diseases, such as chicken pox, provides a natural immunity to those diseases. However, it is possible to protect against many life-threatening diseases, such as typhoid or tetanus, by artificial immunity. Artificial immunity can be acquired by the introduction of antigenic proteins or other molecular antigens into the body in a process called immunization, or by the introduction of whole pathogens, live or rendered harmless, which is called vaccination.

Immunization or vaccination initiates a primary immune response, generating memory cells without making the person ill. Later, if the same or very similar disease organisms attack, B memory cells already exist. They recognize the antigen and quickly overwhelm the invaders with a massive production of lymphocytes and antibodies.

Q Each B cell makes a different specific antibody and displays it on its cell surface.

This cell makes an antibody that binds this specific determinant...



o o o o

/ { / \ / J \

iCl fyt id fii Bl fil ?

^Stf 1 ^s^ \ rssy ^ss> \ ^s^ \ l^ss>'



Plasma cells

/ « K - - ^Antibodies M / T

Memory cells

I Some develop into plasma cells (effector cells) that secrete antibodies binding to the antigenic determinant.

IA few cells divide at a low rate, perpetuating the clone. These are called memory cells.

1 9.7 Clonal Selection in B Cells

The binding of an antigenic determinant to a specific antibody on the surface of a B cell stimulates the cell to divide, rapidly producing a clone of cells to fight the invader.

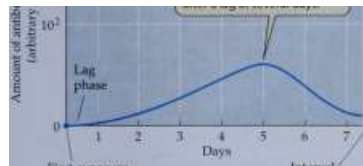
The ability of the human body to remember a specific antigen explains why immunization has almost completely wiped out deadly diseases such as diphtheria and polio in industrialized countries. In fact, smallpox has been eliminated worldwide, thanks to an international effort by the World Health Organization. As far as we know, the only remaining smallpox viruses on Earth are those kept in some laboratories.

Animals distinguish self from nonself and tolerate their own antigens

Given the presence of lymphocytes directed against so many antigens, why doesn't a healthy human produce self-destructive immune responses? The body is tolerant of its own molecules—the same molecules that would generate an immune response in another individual. Self-tolerance seems to be based on two mechanisms: clonal deletion and clonal anergy.

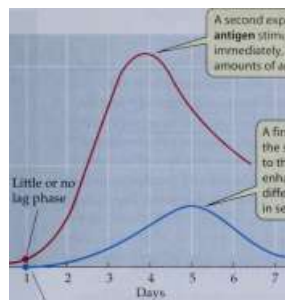
Clonal deletion physically removes B or T cells from the immune system at some point during their differentiation. For example, immature B cells in bone marrow may encounter self antigens. Any of these cells that shows the potential to mount an immune response against self antigens

The first measurable response to an antigen stimulates the body, producing antibodies after a lag of several days.



First exposure to antigen

Interval (perhaps years)



A second exposure to the same antigen stimulates the body to react immediately, producing much larger amounts of antibody.

A first exposure to a different antigen at the same time as the second exposure to the first antigen is not met by an enhanced response. Therefore, a different process (memory) is involved in second response.

79.8 Immunological Memory

The ability of the body to remember an antigen to which it has been exposed is the basis for natural and artificial immunity against a disease.

undergoes programmed cell death (apoptosis) within a short time, and never differentiates enough to make antibodies. Thus, no clones of antiself lymphocytes normally appear in the bloodstream. Clonal deletion eliminates about 90 percent of all the B cells made in the bone marrow. A similar process occurs with T cells in the thymus.

Clonal anergy is the suppression of the immune response. For example, a mature T cell may encounter and recognize a self antigen on the surface of a target cell. But it does not send out the cytokines that signal the initiation of an immune response. Before it does so, the T cell must encounter not only an antigen, but also a second molecule called CD28 on the cell surface. This co-stimulatory signal is expressed only on certain cells called antigen-presenting

4 Days

Second exposure to first antigen; first exposure to second antigen

cells. So most body cells, lacking CD28, will not be attacked by the cellular immune system.

The phenomenon of immunological tolerance (Figure 19.9) was discovered through the observation that some nonidentical twin cattle with different blood types contained some of each other's red blood cells. Why did these "foreign" blood cells not cause immune responses resulting in their elimination? The hypothesis suggested was that the blood cells had passed between the fetal animals in the womb before the lymphocytes had matured. Thus each calf regarded the other's red blood cells as self. This hypothesis was confirmed when it was shown that injecting foreign

79.9 Making Nonself Seem Like Self

The ability of adult mice to recognize and reject grafts of foreign skin can be overcome by earlier exposure to nonself antigens. Both of the mouse strains used in this experiment are highly inbred, and so each member of a strain is genetically identical

to the others in the strain. Strain B mice tolerate grafts from other strain B mice.

EXPERIMENT

Question: When can an animal become tolerant to nonself antigens?



Strain A experimental

METHOD

RESULTS AND INTERPRETATION

mouse



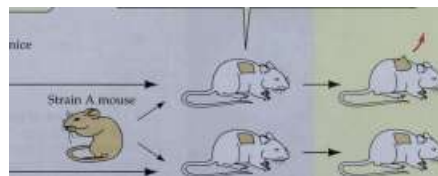
Lymphoid cells from strain A mouse are injected into strain B mice. Strain B control mice are not injected.

8 to 10 weeks later, treated and untreated strain B mice mature into adults, and skin grafts from strain A mice are implanted.

Newborn strain B mice

Control ^ v

Treated



Conclusion: What is recognized as self and nonself depends partly on when it is first encountered.

Control

mouse rejects strain A graft: No tolerance

Treated

mouse accepts strain A graft: Tolerance

362 CHAPTER NINETEEN

antigen into an animal early in fetal development caused that animal henceforth to recognize that antigen as self.

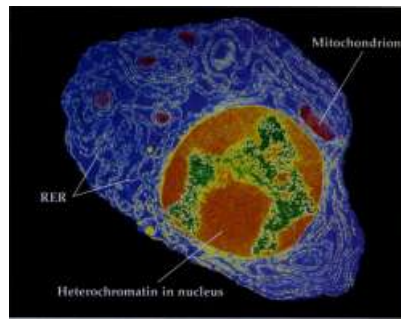
Tolerance must be established repeatedly throughout the life of the animal. Because lymphocytes are produced continuously. Continued exposure to self antigen helps maintain tolerance. For unknown reasons, tolerance to self antigens may be lost. When this happens, the body produces antibodies or T cells against its own proteins, resulting in an autoimmune disease ^ _ ^

B Cells: The Humoral Immune Response

Every day, billions of B cells survive the test of clonal deletion and are released from the bone marrow to enter the circulation. The B cells are the basis for the humoral immune response. Since each B cell expresses on its surface an antibody that is specific for a particular antigen, that antigen can bind to and activate the B cell, causing it to form a clone.

Some B cells develop into plasma cells

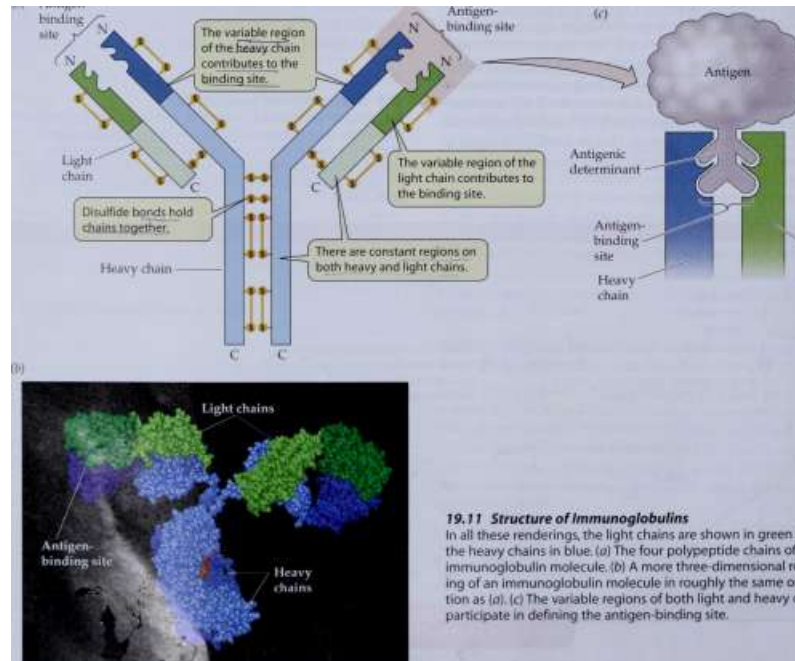
As described above, the activation of a B cell involves the binding of a particular antigenic determinant to the antibody protein on the surface of the B cell. Normally, for such



79.70 A Plasma Cell

The prominent nucleus with large amounts of heterochromatin (orange) and the cytoplasm (bright blue) crowded with rough endoplasmic reticulum are features of a cell that is actively synthesizing and exporting proteins—in this case, antibodies.

{a) Antigen-binding site



Light chain

79.7 7 Structure of Immunoglobulins

In all these renderings, the light chains are shown in green and the heavy chains in blue, (a) The four polypeptide chains of an immunoglobulin molecule, (b) A more three-dimensional rendering of an immunoglobulin molecule in roughly the same orientation as (a), (c) The variable regions of both light and heavy chains participate in defining the antigen-binding site.

NATURAL DEFENSES AGAINST DISEASE 363

For a B cell to develop into antibody-secreting plasma cells, a helper T cell (TH) must also bind to the same antigen on an antigen-presenting cell. The cellular division and differentiation of the B cells is stimulated by the receipt of chemical signals from Th1 and Th2 responsive T cells. These events lead to the formation of plasma cells (the effector cells) and memory cells (see Figure 19.7).

As plasma cells develop, the number of ribosomes and the amount of endoplasmic reticulum in their cytoplasm increase greatly (Figure 19.10). These increases prepare the cells for synthesizing large amounts of antibodies for secretion. All the plasma cells arising from a given B cell produce identical antibodies—specifically

bound to the parent B cell. Thus antibody specificity is maintained as B cells proliferate.

Antibodies share a common structure, but may be of different classes

Antibodies are proteins called immunoglobulins. There are several types of immunoglobulins, but all contain a tetramer consisting of four polypeptides. Two of these polypeptides are identical light chains, and two are identical heavy chains, designated by their different sizes. Disulfide bonds (—S—S—) hold the chains together.

Each polypeptide chain consists of a constant region and a variable region (Figure 19.11). The constant regions of both light and heavy chains are similar in amino acid sequence in the immunoglobulins. The variable regions differ in their amino acid sequences. They contribute directly to the three-dimensional region where the antigen binds—

the antigen-binding site —and are responsible for the diversity of antibody specificity.

The amino acid sequence of the variable region is unique in each of the millions of antigen-specific immunoglobulins. Together the variable regions of a light and a heavy chain form a highly specific, three-dimensional structure. This part of a particular immunoglobulin molecule is what binds with a particular, unique antigenic determinant. The enormous range of antibody specificities is accomplished by a combination of rearrangements and mutations in the genes that encode the variable regions, as we will see later in the chapter.

Although the variable regions are responsible for the specificity of an immunoglobulin, the constant regions are "also - important. The constant regions determine whether the antibody remains part of the cell's plasma membrane or is secreted into the bloodstream. The constant regions also determine the type of action to be taken in eliminating the antigen, as we will see shortly.

The two antigen-binding sites on each immunoglobulin molecule are identical, permitting the formation of a large complex of antigen and antibody molecules. This complex is an easy target for ingestion and breakdown by phagocytic cells.

The five immunoglobulin classes are based on differences in the constant region of the heavy chain ; they are described in Table 19.2.

IgG molecules make up about 85 percent of the total immunoglobulin content of the bloodstream. They are made in greatest quantity during a second immune response (see Figure 19.8). IgG defends the body in several ways. For ex-

Table 19.2 Antibody Classes

CLASS

GENERAL STRUCTURE

LOCATION

FUNCTION

IgG

IgM

IgD

IgA

Monomer

Pentamer

Monomer

Dimer

Y Y

Free in plasma;

about 80 percent

of circulating

antibodies Surface of B cell;

free in plasma

Surface of B cell

Monomer found in plasma; polymers in saliva, tears, milk, and other body secretions

Most abundant antibody in primary and secondary responses; crosses placenta and provides passive immunization to fetus

Antigen receptor on B cell membrane; first class of antibodies released by B cells during primary response

Cell surface receptor of mature B cell; important in B cell activation

Protects mucosal surfaces; prevents attachment of pathogens to epithelial cells

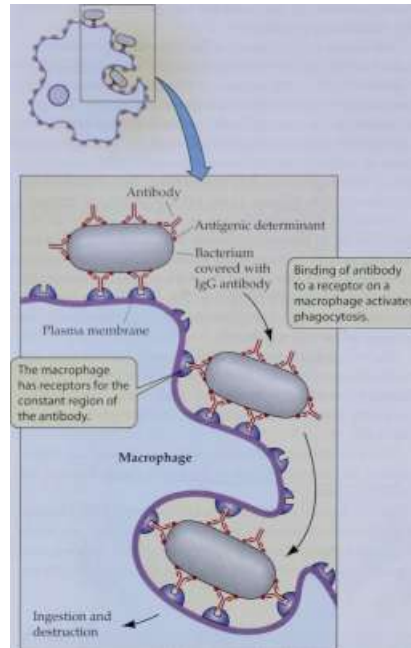
IgE

Monomer

Secreted by plasma cells in skin and tissues lining gastrointestinal and respiratory tracts

Found on mast cells and basophils; when bound to antigens, triggers release of histamine from mast cell or basophil that contributes to inflammation and some allergic responses

364 CHAPTER NINETEEN



79.72 IgG Antibodies Promote Phagocytosis

When IgG antibodies cover a bacterium, receptors on a macrophage recognize them and engulf the cell they have coated.

ample, after some IgG molecules bind to antigens, they become attached by their heavy chains to macrophages. This attachment permits the macrophages to destroy the antigens by phagocytosis (Figure 19.12).

Hybridomas produce monoclonal antibodies

Because most antigens carry many different antigenic

determinants, animals injected with a single antigen will

produce a complex mixture of antibodies. Each of the anti-

bodies is made by a clone of B cells. So the normal antibody

is said to be polyclonal.

We learned in studies of biochemistry that many have regions of similar structure. All human examples have a similar multi-ring structure (Figure 19.24). Some antibodies made by an animal are said to be polyclonal.

Each antigenic determinant has some parts that are

unique to that molecule, and some of the antibodies are directed against this region.

Suppose that a woman is infertile and her physician needs to measure the levels of the hormone estrogen in her blood. This could be done by using an antibody directed against estrogen as a reagent, and seeing how much antigen-antibody complex formed with a sample of the woman's blood. A polyclonal group of antibodies against estrogen would not be useful, however, because some of them, directed against common determinants, would bind to other steroid hormones as well as estrogen. Clearly, a clone of B cells making an antibody that binds only to a unique determinant—a monoclonal antibody—would be needed. But how can that clone be isolated and propagated?

Unfortunately, B cells cannot be cultured. On the other hand, cancerous tumors of plasma cells, called myelomas, grow rapidly in culture. Each tumor arises from a single plasma cell. Some myeloma cells cultured in laboratories have lost the ability to produce antibodies: These cells live for a long time, but they do not secrete immunoglobulins. Scientists use these myeloma cells and normal B lymphocytes to produce hybrid cells called hybridomas, which make specific normal antibodies in quantity and which, like the myeloma cells, proliferate rapidly and indefinitely in culture (Figure 19.13). These clones produce monoclonal antibodies in large quantities and can be preserved and stored by freezing.

Monoclonal antibodies have many practical applications. For example, they have been invaluable in the development of immunoassays, which use the great specificity of the antibodies to detect tiny amounts of molecules in tissues and fluids. This

technique is used to quantify hormones such as estrogen. Most human pregnancy tests use a monoclonal antibody to a hormone made by the developing embryo.

Radioactively tagged monoclonal antibodies are used to target antigens on the surface of cancer cells, enabling precise imaging of the tumor so that the physician can monitor the progress of therapy. The cancer cell-targeted antibody, when attached to a poison, can be used to kill the tumor cells.

Monoclonal antibodies are also used for passive immunization—inoculation with specific antibody rather than with an antigen that causes the patient to develop his or her own antibody (as most vaccines are designed to do). Passive immunization is the approach used to treat the early symptoms of rabies infection or a rattlesnake bite, cases in which the toxic nature of the infection is so serious that there is not enough time to allow the person's immune system to mount its own defense.

T Cells: The Cellular Immune Response

Thus far we have been concerned primarily with the humoral immune response, whose effector molecules are the antibodies secreted by plasma cells that develop from acti-

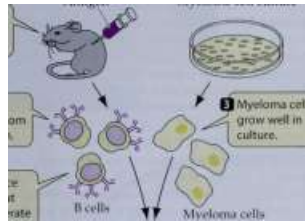
RESEARCH METHOD

A mouse is immunized with antigen.

Antigen

B cells are isolated from the spleen.

B cells produce / ^ antibodies but do not proliferate in culture.



Myeloma cell culture

Myeloma cells grow well in culture.

1

B cells

i

A myeloma cell is fused with a B cell (a hybridoma).

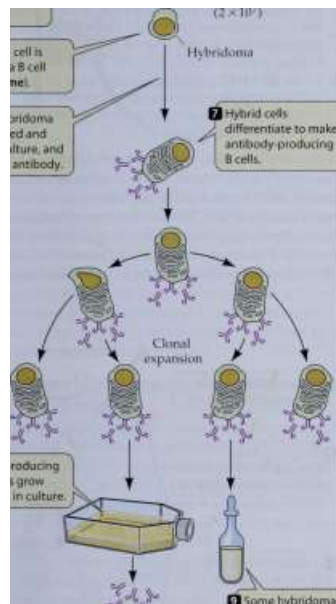
Myeloma cells (2X10⁷)

Hybridoma

A single hybridoma cell is isolated and grown in culture, and assayed for antibody.

Hybrid cells differentiate to make antibody-producing B cells.

^J Antibody-producing hybridomas grow indefinitely in culture



Monoclonal antibodies

Some hybridomas are frozen for future use.

vated B cells. T cells are the effectors of the cellular immune response, which is directed against any factor, such as a virus or mutation, that changes a normal cell into an abnormal cell.

In this section, we will describe two types of T cells (helper T cells and cytotoxic T cells). We will discover that the binding of a T cell receptor to an antigenic determinant re-

19.13 Creating Hybridomas for the Production of Monoclonal Antibodies

Cancerous myeloma cells and normal lymphocytes can be hybridized so that the proliferative properties of the myeloma cells are merged with the properties of the antibody-producing lymphocytes.

quires special proteins encoded by the MHC (major histocompatibility complex) genes. These proteins underlie the immune system's tolerance for the cells of its own body and are responsible for the rejection of foreign tissues by the body.

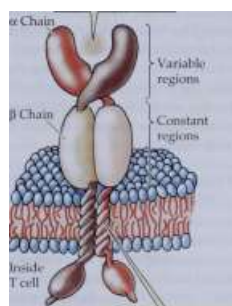
T cell receptors are found on two types of T cells

Like B cells, T cells possess specific surface receptors. T cell receptors are not immunoglobulins, but glycoproteins with molecular weights about half that of an IgG. They are made up of two polypeptide chains, each encoded by a separate gene (Figure 19.14).

The genes that code for T cell receptors are similar to those for immunoglobulins, suggesting that both are derived from a single, evolutionary more ancient group of genes. Like the immunoglobulins, T cell receptors include both variable and constant regions. The variable regions provide the specificity for reaction with a single antigenic determinant.

The antigen and MHC molecules bind here.

a Chain



Inside T cell

A hydrophobic region anchors the chain in the plasma membrane.

19.14 AT Cell Surface Receptor

T cell receptors are glycoproteins, not immunoglobulins, although the structures of the two molecules are similar. The receptors are bound to the plasma membrane of the T cell that produces them.



19.7.5 A Cytotoxic T Cell in Action

A cytotoxic T cell (the smaller sphere) has come into contact with a virus-infected cell, causing the infected cell to lyse. The blisters on the infected cell's surface indicate that it is beginning to break up.

There is a major difference between antibodies and T cell receptors: While antibodies bind to an intact antigen, T cell receptors bind to a piece of the antigen displayed on the surface of an antigen-presenting cell.

When T cells are activated by contact with a specific antigenic determinant, they proliferate and give rise to two types of effector cells:

- Cytotoxic T cells, or T_c cells, recognize virus-infected cells and kill them by causing them to lyse (Figure 19.15).
- Helper T cells, or T_H cells, assist both the cellular and humoral immune systems.

As mentioned already, a specific T_H cell must bind an antigen presented on a B cell before that B cell can become activated. The helper cell becomes the "conductor" of the "immunological orchestra," as it sends out chemical signals that not only result in its own clonal expansion, but also set in motion the actions of cytotoxic T cells as well as B cells.

Now that we are familiar with the major types of T cells, we can address the question of how T cells meet the antigenic determinants.

The major histocompatibility complex encodes proteins that present antigens to the immune system

We have seen that a body's defenses recognize its own cells as self—that proteins on our own cell surfaces are tolerated by our immune systems. There are several types of mammalian cell surface proteins, but we will focus on one very important group, the products of a cluster of genes called the histocompatibility complex, or MHC. Its gene products are plasma membrane glycoproteins with attached carbohydrate groups. In humans, these molecules are called human leukocyte antigens, while in mice they are called H-2 proteins. Their job is to "present" antigens on the cell surface

to a T cell

There are three classes of MHC proteins:

- Class I MHC proteins are present on the surface of every nucleated cell in the animal. When cellular proteins are degraded to small peptide fragments in the proteasome (see Chapter 14), an MHC I protein may bind to a fragment and travel to the plasma membrane. There, the MHC I protein "presents" the cellular peptide to T_c cells. The T_c cells have a surface protein called CD8 that recognizes MHC I.
- Class II MHC proteins are found mostly on the surfaces of B cells, macrophages, and other antigen-presenting cells. When an antigen-presenting cell ingests an antigen, such as a virus, it is broken down in an endosome. An MHC II molecule may bind to one of the fragments and carry it to the cell surface, where it is presented to a T_H cell (Figure 19.16). T_H cells have a surface protein called CD4 that recognizes MHC II.
- Class III MHC proteins include some of the proteins of the complement system that interact with antigen-antibody complexes and result in the lysis of foreign cells (see Figure 19.4).

To accomplish their roles in antigen binding and presentation, both MHC I and MHC II have an antigen-binding groove, which can hold a peptide of about 10–20 amino acids (Figure 19.17). The T cell receptor recognizes not just the antigenic fragment, but the fragment bound to an MHC I or MHC II molecule. The table in Figure 19.17 summarizes the relationships of T cells and antigen-presenting cells.

Q A macrophage takes up T antigen by phagocytosis

§J The macrophage

processes the antigen by breaking it into fragments.

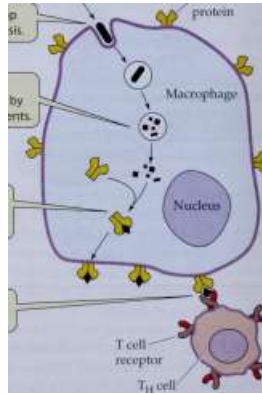
Antigen

Class II MHC \ CVJ protein

Q A class II MHC protein binds the processed antigen.

f

The MHC presents the antigen to the T_H cell



7 9.76 Macrophages Are Antigen-Presenting Cells

Processed antigen is displayed by MHC II protein on the surface of a macrophage. Receptors on the helper T cell can then interact with the processed antigen/MHC II protein complex.

T cells

Antigen-presenting cell

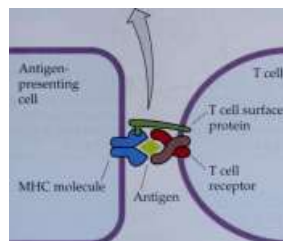
NATURAL DEFENSES AGAINST DISEASE 367

7 9.7 7 The Interaction between T Cells and Antigen-Presenting Cells

A groove in the MHC I protein holds an antigen, which it "presents" to a cytotoxic T cell. CD8 surface proteins on the T cells insure the specificity of interaction.

Antigen-presenting cell

The MHC I protein on the cell's surface has an antigen-binding groove.



Antigen-Presenting and T Cell Types

PRESENTING CELL TYPE

ANTIGEN PRESENTED

MHC CLASS T CELL TYPE

T CELL SURFACE PROTEIN

Any cell

Macrophages and B cells

Intracellular Class I

protein fragment

Fragments from Class II

extracellular

proteins

Cytotoxic T cell CD8

(T_c) T helper cell

(T H) CD4

There are three genetic loci each for MHC I and MHC II, and all six loci have as many as 100 different alleles. With so many possible allelic combinations, it is not surprising that different individuals are very likely to have different MHC genotypes. Similarities in base sequences between MHC genes and the genes coding for antibodies and T cell receptors suggest that all three may have descended from the same ancestral genes and are part of a "superfamily." Major aspects of the immune systems seem to be woven together by a common evolutionary thread.

Helper T cells and MHC II proteins contribute to the humoral immune response

When a T H cell binds to an antigen-presenting macrophage, the T H cell releases cytokines, which activate it to produce a clone of differentiated cells capable of interacting with B cells. The steps to this point constitute the activation phase of the response, and they occur in lymphatic tissue. Next comes the effector phase, in which B cells are activated to produce antibodies (Figure 19.18a).

B cells are also antigen-presenting cells. B cells take up by endocytosis antigen bound to their immunoglobulin receptors, process it, and display it on class II MHC proteins. When a T H cell binds to the displayed antigen-MHC II complex, it releases cytokines that cause the B cell to produce a clone of plasma cells. Finally, the plasma cells secrete antibody, completing the effector phase of the humoral immune response.

Cytotoxic T cells and MHC I proteins contribute to the cellular immune response

Class I MHC molecules play a role in the cellular immune response that is similar to the role played by class II MHC molecules in the humoral immune response. In a virus-infected or mutated cell, "foreign" proteins or peptide frag-

ments combine with MHC class I molecules. The complex is displayed on the cell surface and presented to T c cells. When a T c cell binds to this complex, it is activated to proliferate and differentiate (Figure 19.18b).

In the effector phase of the cellular immune response, T c cells produce molecules that lyse the target cell. In addition, the T c cell can bind to a specific receptor (called Fas) on the target cell, that initiates apoptosis in the target cell. These two mechanisms, cell lysis and programmed cell death, work in concert to eliminate the altered host cell.

Because T cell receptors recognize self MHC molecules complexed with nonself antigens, they help rid the body of its own virus-infected cells. Because they also recognize MHC molecules complexed with altered self antigens (as a result of mutations), they help eliminate tumor cells, since most tumor cells have mutations.

In addition to the binding of an antigen-MHC complex to a cell surface receptor, T cells must receive a second signal for activation. This "co-stimulatory" signal occurs after the initial specific binding, and involves the interaction of additional proteins on the T cell and antigen-presenting cell. This second binding event leads to T cell activation, including cytokine production and cell division. It also sets in motion the production of an inhibitor of these events, so that the response is appropriately terminated. This inhibitor, a cell surface protein called CTLA4, is also important for the acquisition of tolerance, the capacity to avoid attacking one's own antigenic determinants.

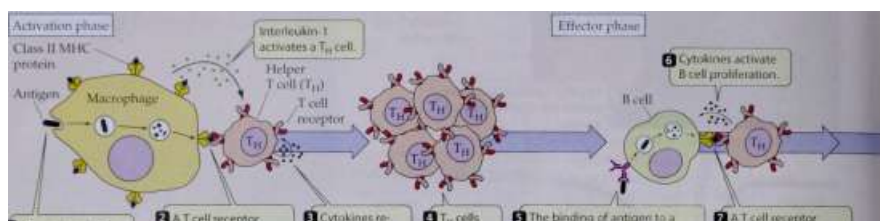
MHC molecules underlie the tolerance of self

MHC molecules play a key role in establishing tolerance to self, without which an animal would be destroyed by its own immune system. Throughout the animal's life, developing T cells are tested in the thymus. One test question is, Can this cell recognize the body's MHC proteins? A T cell

368 CHAPTER NINETEEN

(«)

HUMORAL RESPONSE



jjThe antigen is taken up by phagocytosis and degraded in a lysosome.

Q AT cell receptor recognizes processed antigen bound to a class II MHC protein on the macrophage.

o Cytokines released by the Th cell stimulate it to proliferate.

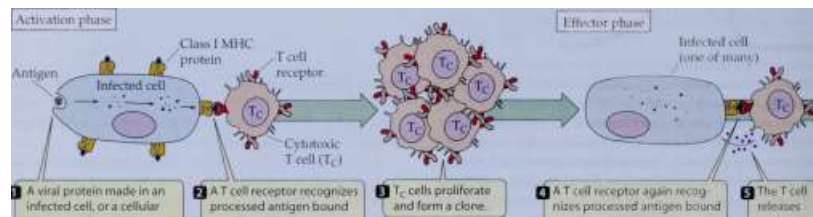
cells proliferate and form a clone.

o The binding of antigen to a specific IgM receptor triggers receptor mediated endocytosis, degradation, and display of the processed antigen.

| A T cell receptor recognizes processed antigen bound to a class II MHC protein on a B cell.

(b)

CELLULAR RESPONSE



J

A viral protein made in an infected cell, or a cellular protein, is degraded to fragments and picked up by a class I MHC protein.

| A T cell receptor recognizes processed antigen bound to a class I MHC protein on an infected cell.

T c cells proliferate and form a clone.

| A T cell receptor again recognizes processed antigen bound to a class I MHC protein.

| The T cell releases perforin.



^ 79.78 Phases of the Humoral and Cellular Immune Responses

Both immune responses have an activation phase and an effector phase.

unable to recognize self MHC proteins would be useless to the animal because it could not participate in any immune reactions. Such a T cell fails the test and dies within about 3 days.

The second question is, Does this cell bind to self MHC protein and to one of the body's own antigens? A T cell that satisfied both of these criteria would be harmful or lethal to the animal; it also fails the test and undergoes apoptosis. T cells that survive these tests mature into either T c cells or T H cells.

MHC molecules are responsible for transplant rejection

In humans, a major consequence of the MHC molecules became important with the development of organ transplant surgery. Because the proteins produced by the MHC are specific to each individual, they act as antigens if trans-

planted into another individual. An organ or a piece of skin transplanted from one person to another is recognized as nonself and soon provokes an immune response; the tissue then is killed, or "rejected," by the cellular immune system. But if the transplant is performed immediately after birth, or if it comes from a genetically identical person (an identical twin), the material is recognized as self and is not rejected.

The rejection problem can be overcome by treating a patient with drugs, such as cyclosporin, that suppress the immune system. However, this approach compromises the ability of patients to defend themselves against bacteria and viruses. Cyclosporin and some other immunosuppressants interfere with communication between cells of the immune system. Specifically, they inhibit the production of cytokines.

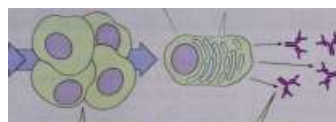
The Genetic Basis of Antibody Diversity

A newborn mammal possesses a full set of genetic information for immunoglobulin synthesis. At each of the 61 loci coding for the heavy and light chains, it has an allele from the mother and one from the father. Throughout the animal's

NATURAL DEFENSES AGAINST DISEASE 369

Plasma Endoplasmic

cell reticulum



t

B cells proliferate and differentiate.

f

The plasma cell produces antibodies.

-

A

t

A

...which lyses the infected cell.

life, each of its cells begins with the same full set of genes. However, as B cells develop, their genomes become modified in such a way that each cell eventually can produce one—and only one—specific type of antibody. In other words, different B cells develop slightly different genomes encoding different antibody specificities. How can a single organism produce millions of different immunoglobulins?

One hypothesis was that we simply have millions of antibody genes. However, a simple calculation (the number of base pairs needed per antibody gene multiplied by millions) shows that if this were true, our entire genome would be taken up by antibody genes. More than 30 years ago, an alternative hypothesis was proposed: A relatively small number of genes recombine at the DNA level to produce many unique combinations, and it is this shuffling of the genetic deck that produces antibody diversity. This is now the accepted theory.

In this section, we will describe the unusual events that generate the enormous antibody diversity normally characterizing each individual mammal. Then we will see how similar events produce the five classes of antibodies by producing slightly different "constant regions" that have special properties.

Antibody diversity results from

DNA rearrangement and other mutations

In an unusual genetic process, functional immunoglobulin genes are assembled from DNA segments that initially are spatially separate. Every cell in the body has hundreds of DNA segments potentially capable of participating in the synthesis of the variable regions of the Ig molecule. In most body cells, these DNA sequences remain intact and separate from one another. During B cell development, however, these DNA segments are rearranged and joined. Pieces of the DNA are deleted, and DNA segments formerly distant from one another are joined together. In this fashion, an immunoglobulin gene is assembled from randomly selected pieces of DNA. Each B cell has its own unique set of immunoglobulin genes. This remarkable process generates many diverse antibodies from the same starting genome. The same type of process also accounts for the diversity of T cell receptors.

In both humans and mice, the DNA segments coding for immunoglobulin heavy chains are on one chromosome and those for light chains are on others. The variable region of the light chain is encoded by two families of DNA segments, and the variable region of the heavy chain is encoded by three families.

Look at Figure 19.19 for an example of the gene families coding for the constant and variable regions of the heavy chain in mice. There are multiple genes coding for each of four kinds of segments in the polypeptide chain: 100 V, 30 D, 6 J, and 8 C. Each B cell that becomes committed to making an antibody randomly selects one gene for each of these segments to make the final heavy-chain coding sequence, VDJC. So the number of different heavy chains that can be made through this random recombination process is quite large.

Now consider that the light chains are similarly constructed, with a similar amount of diversity made possible by random recombination. If we assume that light-chain diversity is the same as heavy-chain diversity (144,000 possibilities), the number of possible combinations of light and heavy chains is 144,000 different light chains \times 144,000 different heavy chains = 21 billion possibilities!

Even if this number is an overestimate by severalfold (and it is), the number of different immunoglobulin molecules that a B cell can make is huge. But there are still other mechanisms that generate even more diversity:

► When the DNA sequences for the V, J, and C regions are rearranged so that they are next to one another, the recombination event is not precise, and errors occur at the junctions. This imprecise recombination can create new codons at the junctions, with resulting amino acid changes.

► After the DNA fragments are cut out and before they are joined, an enzyme, terminal transferase, often adds some nucleotides to the free ends of the DNA's. These additional bases create insertion mutations.

► Finally, there is a relatively high mutation rate in immunoglobulin genes. Once again, this process creates many new alleles and antibody diversity.

370 CHAPTER NINETEEN

Segments encoding variable region (V)

V_H V_D V_J ...V.

100

(variable) segments

2 3 4.

nm

.100 TT -

1

11111111

Q The variable region for the heavy chain of a

particular antibody is encoded by one K segment, one D segment, and one J segment. Each of these segments is taken from a pool of like segments.

7 9.7 9 Heavy-Chain Genes

Mouse immunoglobulin heavy chains have four segments, each of which is coded for by one of multiple genes.

Adding these possibilities to the billions of combinations that can be made by random DNA rearrangements makes it not surprising that the immune system can mount a response to almost any natural or human-made substance.

How does a B cell produce a specific heavy chain?

As an example of how DNA rearrangement generates antibody diversity, let's consider how the heavy chain of IgM is produced in the mouse, a favorite subject for immunology studies.

The gene families governing all heavy-chain synthesis are on mouse chromosome 12, with the members arranged as shown in Figure 19.19. Light chains are produced from similar families, but they lack D segments.

How does order emerge from this seeming chaos of DNA segments? Two distinct types of nucleic acid rearrangements contribute to the formation of an antibody:

D, D 2 ... D 3 (J) 2 ... J b (diversity) (joining) segments segments

30

1...6

Segments encoding constant region (C) a

u5

y3

yl y2b y2a e

a

TT

tThe constant region is selected from another pool of segments.

§J The number of possible combinations to make an immunoglobulin from this set of genes is:

$$(100 V)/(30 D)(6 J)(8 C) = 144,000$$

► DNA rearrangements, before transcription, join the V, D, and / segments.

► RNA splicing, after transcription, joins the variable region (VDJ) to the constant region.

First, substantial chunks of DNA are deleted from the chromosome during rearrangement of the segments. As a result of these deletions, a particular D segment ends up directly beside a particular / segment, and then the DJ segment ends up adjacent to one of the V segments. Thus, a single

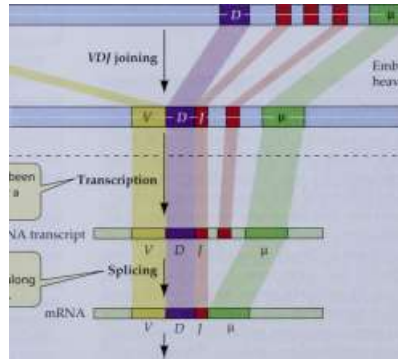
(a) DNA rearrangement

Embryonic DNA

/ segments

C segments

B cell DNA



Embryonic μ gene for heavy chain of IgM

(b) RNA splicing

O After V, D, J, and C DNA segments have been joined, the resulting functional gene for a heavy chain is transcribed.

Primary RNA transcript

Q Splicing of the primary RNA transcript

removes the transcripts of any introns, along with transcripts of any extra J segments.

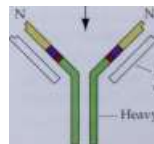


79.20 Heavy-Chain Gene Rearrangement and Splicing

Two t, of rearrangement in the heavy-chain gene are required for an formation, (a) Prior to transcription, DNA is rearranged to jo the V, D, and J segments into a variable region. (b) After transcription, RNA splicing joins the VDJ region to the constant region.

Translation of immunoglobulin heavy chains

Light chain



Heavy chain

C C

"new" sequence, consisting of one V, one D, and one / segment, can now code for the variable region of the heavy chain. All the progeny of this cell constitute a clone having the same sequence for the variable region (Figure 19.20a).

The second step follows transcription. Splicing of the RNA transcript removes introns and any / segments lying between the selected / segment and the first constant region segment (Figure 19.20/?). The result is an mRNA that can be translated, directly yielding the heavy chain of the cell's specific antibody.

The constant region is involved in class switching

In Table 19.2, we described the different classes of antibodies and their functions. Generally, a B cell makes only one class at a time. But class switching can occur, in which a B cell changes which antibody class it synthesizes.

Early in its life, a B cell produces IgM molecules, which are responsible for the specific recognition of a particular antigenic determinant. At this time, the constant region of the antibody's heavy chain is encoded by the first constant region segment, the μ segment (see Figure 19.19). If the B cell later becomes a plasma cell during an immunological response, another deletion commonly occurs in the cell's DNA, positioning the heavy-chain variable region gene (consisting of the same V, D, and / segments) next to a constant region segment farther down the original DNA, such as the γ , δ , or α segments (Figure 19.21). Such a DNA deletion results in the production of an antibody with a differ-

DNA [V



| This VDJC gene was J* formed by DNA rearrangements.

DNA

IgG gene

3

DNA rearrangement

V

72b—72a—e—a-

Transcription and splicing

mRNA

V D] Y2b

\

Deletion of part of the constant region causes a new C gene to be expressed (IgG instead of IgM).

Translation

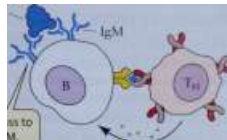
19.21 Class Switching

The gene produced by joining V,D,J, and C segments (see Figure19.20) may later be modified, causing a different C segment to be transcribed. This modification, known as class switching, is accomplished by deletion of part of the constant region. Shown here is class switching from an IgM gene to an IgG gene.

f

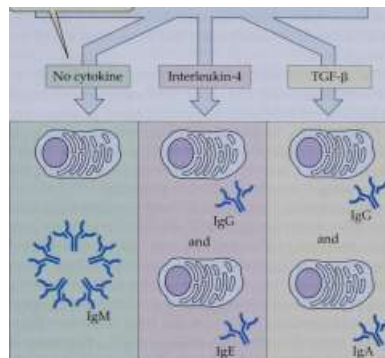
Antigen

The first lg class to be made is IgM.



Cytokines determine which new lg class will be made.

B cell activation



1 9.22 Cytokines Determine How the Antibody Switches Class

AT H cell initiates class switching in a B cell by secreting a cytokine. Different cytokines produce different switches.

ent constant region and function, but the same antigen specificity. The new antibody has the same variable regions of the light and heavy chains, but a different constant region of the heavy chain. This new antibody falls into one of the four other immunoglobulin classes (IgA, IgD, IgE, or IgG), depending on which of the constant region segments is placed adjacent to the variable region gene.

After switching classes, the plasma cell cannot go back to making the previous immunoglobulin class, because that part of the DNA has been lost. On the other hand, if additional constant region segments are still present, the cell may switch classes again.

What triggers class switching, and what determines the class to which a given B cell will switch? T H cells direct the course of an antibody response and determine the nature of the attack on the antigen. These T cells induce class switching by sending cytokine signals (Figure 19.22). These signals bind to receptors on the target B cells, generating a signal transduction cascade and resulting in altered transcription of immunoglobulin genes.

372 CHAPTER NINETEEN

The Evolution of Animal Defense Systems

The strategy of rearranging DNA to make variable antibodies is used by all vertebrates with jaws, but nowhere else in the animal world. It is an "anticipatory" strategy, since the organism makes not only the defensive proteins that it need-- , but also the antibodies and T cell receptors that it might need. Therefore, it must have provided an evolutionary advantage to the organisms that first had it.

This anticipatory strategy may first have arisen in an ancient creature resembling today's sharks, when a transposon inserted itself into a gene used for a defensive protein. Over time, this inserted element developed the ability to cut out adjacent DNA sequences and move them elsewhere in the genome.

The invertebrates, in all their diversity, have sturdy innate defense systems, and certain defense system elements are found even in unicellular protists. Many protists carry on phagocytosis, as do our own macrophages, and some protists use phagocytosis as a defense mechanism. Multicellular animals, both invertebrate and vertebrate, employ mobile phagocytic cells to patrol their bodies.

Like vertebrates, invertebrates (and probably some protists) distinguish between self and nonself. Making such distinctions enables invertebrates to reject tissue grafted from other individuals of the same species. Unlike vertebrates, however, invertebrates reject a second graft no more rapidly than a first graft—indicating that invertebrates lack immunological memory. This and other observations show that although immunological functions of invertebrates and vertebrates may be similar, their mechanisms often differ.

Invertebrates do not produce immunoglobulins, lymphocytes, or the complement system. However, they achieve similar defensive goals by analogous methods, and the analogs are probably evolutionary precursors of the systems found in vertebrates. Many invertebrates make proteins very similar to vertebrate cytokines, and those proteins play regulatory roles similar to those in humans.

Disorders of the Immune System

Immune deficiency diseases such as AIDS show us how much we depend on our immune system to protect us from pathogens. However, sometimes the immune system fails us in one way or another. It may overreact, as in an allergy; it may attack self antigens, as in an autoimmune disease; or it may function weakly or not at all, as in an immune deficiency disease. After a look at allergies and autoimmune conditions, we will examine the acquired immune deficiency that characterizes AIDS.

An inappropriately active immune system can cause problems

hypersensitivity. A common type of condition can arise when the human immune system overreacts to a dose of antigen (hypersensitivity). Although the antigen itself may present no danger to the host, the inappropriate immune

response may produce inflammation and other symptoms that can cause serious illness or death. Allergies are the most familiar examples of such a problem.

There are two types of allergic reactions. Immediate hypersensitivity occurs when an individual makes large amounts of IgE that bind to a molecule or structure in a food, pollen, or the venom of an insect. Mast cells in tissues and basophils in blood bind the IgE, which causes them to release amines such as histamine. The result is symptoms such as dilation of blood vessels, inflammation, and difficulty breathing. If not treated with antihistamines, a severe allergic reaction can lead to death.

Delayed hypersensitivity does not begin until hours after exposure to an antigen. In this case, the antigen is processed by antigen-presenting cells and a T cell response is initiated. This response can be so massive that the cytokines released cause macrophages to become activated and damage tissues. This is what happens when the bacteria that cause tuberculosis colonize the lung.

autoimmunity. Sometimes clonal deletion fails, resulting in the appearance of one or more "forbidden clones" of B and T cells directed against self antigens (autoimmunity). This failure does not always result in disease, but in some instances it can.

People with systemic lupus erythematosus (SLE) have antibodies to many cellular components, including DNA and nuclear proteins. These antinuclear antibodies can cause serious damage when they link up with normal tissue antigens to form circulating immune complexes, which become stuck in tissues and provoke inflammation. A person with SLE has hyperactive B cells (thus the excess antibodies).

A person with rheumatoid arthritis has difficulty in shutting down a T cell response. We mentioned earlier that the inhibitor CTLA4 blocks T cells from reacting to self antigens. People with rheumatoid arthritis may have low CTLA4 activity, which

results in inflammation of joints due to the infiltration of excess white blood cells.

Multiple sclerosis usually affects young adults, causing progressive damage to the nervous system. It involves both T cell- and B cell-mediated attack on two major proteins in myelin, the special membrane that coats some nervous tissues.

Insulin-dependent diabetes mellitus, or type I diabetes, occurs most often in children. It involves an immune reaction against several proteins in the cells of the pancreas that manufacture the protein hormone insulin. This reaction results in the cells being killed. These patients must take insulin daily in order to survive.

The causes of these autoimmune diseases are not known. They tend to "run in families," indicating a genetic component. Some alleles of MHC II are strongly linked to certain of these diseases. In some cases, the underlying reason may be molecular mimicry, in which T cells that recognize a non-self antigen also recognize something on the self that has a similar structure.

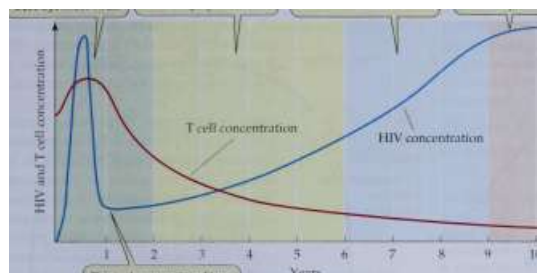
Q Soon after the initial HIV infection, the immune system destroys most virus.

E1 The T cell concentration falls and HIV concentration rises, accompanied by symptoms such as swollen lymph nodes

NATURAL DEFENSES AGAINST DISEASE 373

SJ As T cells are further reduced, immune function is impaired and opportunistic infections occur.

Q Finally, almost all natural immunity is lost.



This is the set point of low-level HIV production.

AIDS is an immune deficiency disorder

There are various immune deficiency disorders, such as those in which T or B cells never form, and others in which B cells lose the ability to give rise to plasma cells. In either case, the affected individual is unable to mount an immune response and thus lacks a major line of defense against microbial pathogens.

Because of its essential roles in both the humoral and cellular immune responses, the T H cell is perhaps the most central of all the components of the immune system—a significant cell to lose to an immune deficiency disorder. This cell is the target of HIV (human immunodeficiency virus), the virus that eventually results in AIDS (acquired immune deficiency syndrome).

HIV is transmitted from person to person several ways:

- ▶ Through blood, such as by an injection needle contaminated with the virus after being used by an infected individual
- ▶ Through exposure of broken skin, an open wound, or mucous membranes to body fluids, such as semen, containing HIV
- ▶ Through the blood of an infected mother to her baby during birth

HIV initially infects macrophages, T M cells, and dendritic cells in blood and tissues. Dendritic cells are antigen-presenting cells with highly folded plasma membranes that can capture antigens (see Figure 19.2). These infected cells carry the virus to the lymphoid tissues (lymph nodes and spleen) where B cells mature and T cells reside.

Normally in the lymph node, the dendritic cells present their captured antigen to T H cells, and this causes the T H cells to divide and form a clone (see Figure 19.18). But HIV preferentially infects activated, and not resting, T H cells. So the HIV arriving in the lymph nodes proceeds to infect the many activated T n cells that are already responding to other

-having cells take the virus

antigens. These two processes

7 9.23 The Course of an HIV Infection

HIV infection may be carried, unsuspected, for many years before the onset of symptoms. This long "dormant" period means the infection is often spread by people who are unaware that they are carrying the virus.

to the nodes and having cells in the nodes already receptive to virus infection—combine to ensure that HIV reproduces vigorously. Up to 10 billion viruses are made every day during this phase. The numbers of T H cells quickly drop, and people show symptoms similar to mononucleosis, such as enlarged lymph nodes and fever.

These symptoms abate within 3 weeks, however, when T cells recognize infected lymphocytes, an immune response is mounted, and antibodies appear in the blood (Figure 19.23). By this time (several months after initial infection) the patient has a lot of circulating HIV complexed with antibodies that is gradually removed by the action of dendritic cells. But before they are filtered out, these antibody-complexed viruses can still infect T H cells that come in contact with them. This secondary infection process reaches a low, steady-state level called the "set point." This point varies between people, and is a strong predictor of the rate of progression of the disease. For most people, it takes 8-10 years, even if untreated, for the more severe manifestations of AIDS to develop. In some, it can take as little as a year; in others, 20 years. During this "incubation period," infected people generally feel fine, and their T H cell levels are adequate for them to mount immune responses.

However, in time, the virus destroys the T H cells, and their numbers fall to dangerous levels. At this point, the infected patient is considered to have full-blown AIDS, and is susceptible to infections that the T H cells would normally have been involved in eliminating (Figure 19.24). Most notable are the otherwise rare skin tumor called Kaposi's sarcoma caused by a herpesvirus, pneumonia caused by the fungus *Pneumocystis carinii*, and lymphoma tumors caused by Epstein-Barr virus. These are called opportunistic infections, because they take advantage of the crippled immune system of the host. They lead to death within a year or two.

HIV infection and replication occur in T H cells

As a retrovirus, HIV uses RNA as its genetic material. A central core, with a protein coat, contains two identical molecules of RNA as well as the enzymes reverse transcriptase, integrase, and a protease. An envelope, derived from the plasma membrane of the cell in which the virus was formed, surrounds the core. The envelope is studded with envelope proteins (gp120 and gp41, where "gp" stands for glycoprotein) that enable the virus to infect its target cells. The HIV replication cycle has several stages.

374 CHAPTER NINETEEN

Swollen lymph nodes

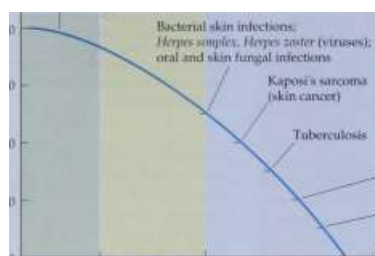
800

T3 O

£ 600

a 200 -

X



400 -

Bacterial skin infections;

Herpes simplex, Herpes zoster (viruses);

oral and skin fungal infections

Kaposi's sarcoma (skin cancer)

Tuberculosis

2 years

6 years

1 9.24 Relationship Between T H Cell Count and Opportunistic Infections

As HIV kills more and more T H cells, the immune system is less and less able to defend the body against various diseases.

virus entry into cells. HIV attaches to T H cells and macrophages via CD4, which acts as a receptor for the viral envelope protein gp120. Following binding, the membrane that surrounds the HIV core particle fuses with the host cell plasma membrane, resulting in the entry of the core into the cytoplasm (Figure 19.25; see also Figure 13.7). These events require the participation of at least two other membrane proteins, one from the virus (gp41) and another from the host (appropriately called fusin in T H cells).

reverse transcription. HIV can insert a cDNA copy of its genome into the host cell's DNA. The process of making a cDNA copy from viral RNA occurs in the viral core particle in an infected cell. It requires the participation of three separate enzymes:

- ▶ Reverse transcriptase, to make single-stranded DNA from an RNA template
- ▶ RNase H, to degrade the viral RNA
- ▶ DNA polymerase, to make the second strand of cDNA

HIV reverse transcriptase does not have the proofreading activity of many DNA polymerases, so the errors that inevitably creep into the process are not corrected. Up to 10 incorrect bases out of about 8,000 may end up in each cDNA produced. This is a great advantage to the virus, as genomic mutations allow its proteins to escape the host's immune response; however, the mutations present a challenge to scientists trying to design drugs and vaccines to bind to the constantly changing viral proteins.

integration of viral cDNA into the host genome. The

viral core proteins have an amino acid sequence that is recognized by a receptor on the surface of the host cell's nucleus; thus the particle rapidly enters the nucleus. At this point, a viral enzyme called integrase catalyzes the breakage of host DNA and insertion of viral cDNA. This is similar to

Pneumocystis carinii pneumonia

Cytomegalovirus infection; lymphoma (tumor)

10 years

the way in which bacteriophage DNA becomes incorporated into a bacterial chromosome as a prophage (see Chapter 13). The cDNA thus becomes a permanent part of the T H cell's DNA, replicating with it at each cell division, and may remain in the T H cell genome for a decade or more.

virus production. This latent period ends if the HIV-infected T H cell becomes activated as it responds naturally to an antigen. The expression of viral genes requires the collaboration of host transcription factors that are made in activated T H cells and a virally encoded protein called Tat. When the T H cell is activated, the entire viral genome is transcribed into RNA, which can either remain as it is or be spliced. Unspliced RNA's become the genomes of new HIV particles; spliced RNA's make the viral structural proteins. An important activator of splicing is the viral protein called Rev.

The protease encoded by HIV is needed to complete the formation of individual viral proteins from larger products of translation. Packaging domains on viral proteins cause the RNA genomes to fold into them and form core particles. In the meantime, the viral membrane proteins are made on the endoplasmic reticulum of the host cell and transported to the plasma membrane via the Golgi complex. The cytoplasmic tails of the gp120 membrane proteins bind to the core particles, and the viruses bud from the infected cell, surrounding themselves with modified plasma membrane from the host.

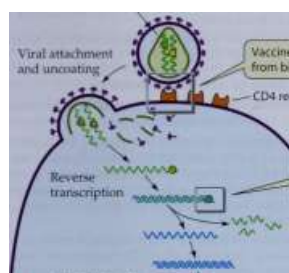
Treatments for HIV infection rely on knowledge of its molecular biology

As the AIDS epidemic has grown, so has knowledge of HIV molecular biology. The general therapeutic strategy is to try to block a stage in the viral life cycle and hold HIV infection in check. Potential therapeutic agents that interfere with the major steps of the life cycle are being tested (see Figure 19.25). Of course, it is crucial to block only steps that are unique to the virus, so that drug therapies do not harm the patient by blocking a step in the patient's own metabolism. Highly active flintiretroviral therapy (HAART) was developed in the late 1990s and has had considerable success in delaying the onset of AIDS symptoms in people infected with HIV by 3 years or more, and in prolonging the lives of people with AIDS. The logic of HAART comes from cancer treatment: Employ a combination of drugs acting at different parts of the virus life cycle. Generally, the HAART regimen uses:

- ▶ A protease inhibitor. These drugs obstruct the active site of the HIV protease.
- ▶ Two reverse transcriptase inhibitors. These molecules are incorporated into the growing cDNA chain, but no nucleotides can be added to them, so reverse transcription stops.

HIV virus

Viral attachment and uncoating



Vaccines may prevent HIV from binding to CD4.

CD4 receptor protein

AZT, ddI, and ddC inhibit reverse transcription.

W[^]

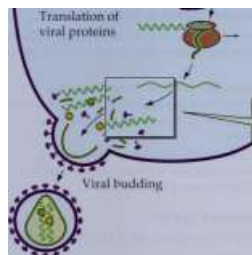
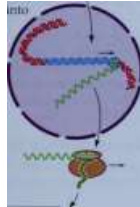
WWWWV

\

\

Integration into host DNA

Translation of viral proteins



Protease inhibitors block the production of mature, functional viral proteins.



^ 79.25 Strategies to Combat HIV Reproduction

Several widely used drugs block specific steps in the HIV life cycle.

These drugs have had such dramatic effects on patients that they may eliminate HIV entirely in some people, especially in those treated within the first few days after infection, before the virus has arrived in the lymph nodes. Most patients, however, face a lifetime of anti-HIV therapy. The many treatments under development include:

- ▶ Vaccines to inhibit virus entry into cells and to form immune complexes with circulating viruses
- ▶ Integrase inhibitors, to block cDNA incorporation into the host genome
- ▶ Tat and Rev inhibitors, to block HIV transcription and splicing
- ▶ Antisense RNA and ribozymes directed against HIV RNA

What can be done until biomedical science provides the tools to bring the worldwide AIDS epidemic to an end? Above all, people must recognize that they are in danger

whenever they have sex with a partner whose total sexual history is not known. The danger rises as the number of sex partners rises, and the danger is much greater if partners participating in sexual intercourse are not protected by a latex condom. The danger that heterosexual intercourse will transmit HIV rises tenfold to a hundredfold if either partner has another sexually transmitted disease.

Chapter Summary

- ▶ Animals defend themselves against pathogens by both innate (nonspecific) and specific means.

Defensive Cells and Proteins

- ▶ Many of our defenses are implemented by cells and proteins carried in the bloodstream and in the lymphatic system. Review Figure 19.1

- ▶ White blood cells, including lymphocytes (B and T cells) and phagocytes (such as neutrophils and macrophages), play many defensive roles. Review Figure 19.2

- ▶ Immune system cells produce several kinds of proteins. Antibodies and T cell receptors bind foreign substances, MHC

proteins help coordinate the recognition of foreign substances and the activation of defensive cells, and cytokines alter the behavior of other cells.

Innate Defenses

- ▶ An animal's innate defenses include physical barriers, competing resident microorganisms, and local agents, such as secretions that contain an antibacterial enzyme. Review Table 19.1
- ▶ The complement system, composed of about 20 proteins, assembles itself in a cascade of reactions to cooperate with phagocytes or antibodies to lyse foreign cells. Review Figure 19.3
- ▶ Interferons produced by virus-infected cells inhibit the ability of viruses to replicate in neighboring cells.
- ▶ Macrophages and neutrophils engulf invading bacteria. Natural killer cells attack tumor cells and virus-infected body cells.
- ▶ Macrophages play an important role in the inflammation response. Activated mast cells release histamine, which causes blood capillaries to leak. Complement proteins attract macrophages to the site, where they engulf bacteria and dead cells. Review Figure 19.5

Specific Defenses:The Immune Response

- ▶ Four features characterize the immune response: specificity, the ability to respond to an enormous diversity of antigens, the ability to distinguish self from nonself, and memory.
- ▶ The immune response is directed against antigens that evade the nonspecific defenses. Each antibody or T cell is directed against a particular antigenic determinant. Review Figure 19.6
- ▶ The immune response is highly diverse and can respond specifically to millions of different antigenic determinants.
- ▶ The immune response distinguishes its own cells from foreign cells, attacking only cells recognized as nonself.
- ▶ The immune system remembers; it can respond rapidly and effectively to a second exposure to an antigen.
- ▶ There are two interactive immune responses: the humoral immune response and the cellular immune response. The humoral immune response employs antibodies secreted by

376 CHAPTER NINETEEN

plasma cells to target antigens in body fluids. The cellular immune response employs 1 cells to attack body cells that have been altered by viral infection or mutation or to target antigens that have invaded the body's cells.

- ▶ Clonal selection theory accounts for the rapidity, specificity, and diversity of the immune response. It also accounts for immunological memory, which is based on the production of both effector and memory cells as T cell and B cell clones expand. Review Figure 19.7
- ▶ Immunological memory plays roles in both natural immunity and artificial immunity based on vaccination. Review Figure 19.8
- ▶ Clonal selection theory also accounts for the immune system's recognition of self. Tolerance of self results from clonal deletion of antiself lymphocytes. Review Figure 19.9

B Cells:The Humoral Immune Response

- ▶ Activated B cells form plasma cells, which synthesize and secrete specific antibodies.
- ▶ The basic unit of an antibody, or immunoglobulin, is a tetramer of four polypeptides: two identical light chains and two identical heavy chains, each consisting of a constant and a variable region. Review Figure 19.11
- ▶ The variable regions of the light and heavy chains collaborate to form the antigen-binding sites of an antibody. The variable regions determine an antibody's specificity; the constant region determines the destination and function of the antibody.
- ▶ There are five immunoglobulin classes. IgM, formed first, is a membrane receptor on B cells, as is IgD. IgG is the most abundant antibody class and performs several defensive functions. IgE takes part in inflammation and allergic reactions. IgA is present in various body secretions. Review Table 19.2
- ▶ Monoclonal antibodies consist of identical immunoglobulin molecules directed against a single antigenic determinant. Hybridomas are produced by fusing B cells with myeloma cells (from cancerous tumors of plasma cells). Review Figure 19.13

T Cells:The Cellular Immune Response

- ▶ The cellular immune response is directed against altered or antigen-infected cells of the body. T c cells attack virus-infected or tumor cells, causing them to lyse. T H cells activate B cells and influence the development of other T cells and macrophages. Review Figure 19.14

- ▶ T cell receptors in the cellular immune response are analogous to immunoglobulins in the humoral immune response.
- ▶ The major histocompatibility complex (MHC) encodes many membrane proteins. MHC molecules in macrophages, B cells, or body cells bind processed antigen and present it to T cells. Review Figure 19.16,19.17
- ▶ The activation of the humoral immune response requires the collaboration of class II MHC molecules, the T cell surface protein CD4, and cytokines. The effector phase of the humoral immune response involves T cells, class II MHC molecules, B cells, and cytokines, and results in the formation of active plasma cells. Review Figure 19.18
- ▶ In the cellular immune response, class I MHC molecules, T c cells, CD8, and cytokines collaborate to activate T c cells with the appropriate specificity. Review Figure 19.18
- ▶ Developing T cells undergo two tests: They must be able to recognize self MHC molecules, and they must not bind to both self MHC and any of the body's own antigens. T cells that fail either of these tests die.
- ▶ The rejection of organ transplants results from the genetic diversity of MHC molecules.

The Genetic Basis of Antibody Diversity

- ▶ Several gene families underlie the incredible diversity of antibody and T cell receptor specificities.
- ▶ Antibody heavy-chain genes are constructed from one each of numerous V, D, J, and C segments. The V, D, and / segments combine by DNA rearrangement, and transcription yields an RNA molecule that is spliced to form a translatable mRNA. Other gene families give rise to the light chains. Review Figures 19.19,19.20
- ▶ There are millions of possible antibodies as a result of these DNA combinations. Imprecise DNA rearrangements, mutations, and random addition of bases to the ends of the DNA's before they are joined contribute even more diversity.
- ▶ A plasma cell produces IgM first, but later it may switch to the production of other classes of antibodies. This class switching, resulting in antibodies with the same antigen specificity but a different function, is accomplished by cutting and rejoining of the genes encoding the constant region. Review Figure 19.21,19.22

The Evolution of Animal Defense Systems

- ▶ The DNA rearrangement mechanism for creating diversity among immune system molecules may have evolved from a DNA transposon.
- ▶ Invertebrate animals reject nonself tissues but lack immunological memory. They possess cells and molecules analogous, but not identical, to lymphocytes, immunoglobulins, and cytokines.
- ▶ Even the most evolutionary ancient groups among today's vertebrates have immune systems more similar to those of humans than to those of invertebrates.

Disorders of the Immune System

- ▶ Allergies result from an overreaction of the immune system to an antigen.
- ▶ Autoimmune diseases result from a failure in the immune recognition of self, with the appearance of antiseif B and T cells that attack the body's own cells.

▶ Immune deficiency disorders result from failures of one or another part of the immune system. AIDS is an immune deficiency disorder arising from depletion of the body's T H cells as a result of infection with HIV. Depletion of the T H cells weakens and eventually destroys the immune system, leaving the host defenseless against "opportunistic" infections. Review Figures 19.23, 19.24

▶ HIV inserts a copy of its genome into a chromosome of a macrophage or T H cell, where it may lie dormant for years. When the viral genome is transcribed and translated, new viruses form. Review Figure 19.25

▶ All steps in the reproductive cycle of HIV are under investigation as possible targets for drugs. Currently the most effective drugs are those directed against reverse transcriptase and protease. Review Figure 19.25

▶ Some treatments may provide a dramatic reduction in HIV levels, but there is as yet no indication that we can prevent infection with HIV, as by vaccination. The only strategy currently available is for people to avoid behaviors that place them at risk.

NATURAL DEFENSES AGAINST DISEASE 377

For Discussion

1. Describe the part of an antibody molecule that interacts with an antigenic determinant. How is it similar to the active site of an enzyme? How does it differ from the active site of an enzyme?

2. Contrast immunoglobulins and T cell receptors with respect to structure and function.
3. Discuss the diversity of antibody specificities in an individual in relation to the diversity of enzymes. Does every cell in an animal contain genetic information for all the organism's enzymes? Does every cell contain genetic information for all the organism's immunoglobulins?
4. The gene family determining MHC on the cell surface in humans is on a single chromosome. A father's MHC type is A1, A3, B5, B7, D9, D11. His wife's phenotype is A2, A4, B6, B7, D11, D12. They have a child who is A1, A4, B6, B7, D11, D12. What are the parents' haplotypes—that is, which alleles are linked on the diploid chromosomes in each parent? Assuming that there is no recombination among the genes determining the MHC type, can these parents have a child who is A1, A2, B7, B8, D10, D11?
5. Is it true that any child can accept an organ transplant from either parent, but parents cannot accept a graft from a child? Explain your answer.

Part Three

Evolutionary Processes



& *.•••Tval



The History of Life on Earth



When you want to know what time it is, you probably look at your watch, or at the clock on the wall or on your computer. You could also listen to the radio or watch television to hear some announcement of time. But suppose the electric power system failed and you lost your watch. How could you tell time then? You would use the cues that people used during most of human history—the cycle of day and night. We are so accustomed to having time-measuring devices all around us that we forget these devices are recent inventions. When Galileo studied the motion of a ball rolling down an inclined plane 350 years ago, he used his pulse to mark off equal intervals of time.

The science of biology is intimately linked to concepts of time. Biology as we know it could not and did not develop very far until an appreciation of the age of Earth was provided by geologists more than 150 years ago. Until that time, most people believed that Earth was only a few thousand years old. Darwin could not have developed his theory of evolution by natural selection if he had not read the works of Charles Lyell, England's leading geologist, who believed that Earth was ancient. As we pointed out in Chapter 1, Darwin's theory was based on the assumption that Earth was very old and that life had existed for a very long time, during which it had steadily evolved.

The goals of Part Three are to document the history of life on Earth, to describe the processes of evolutionary change, and to discuss the agents that cause them. We begin in this chapter by asking the following questions: How do we know that Earth is ancient? What is the evidence that life evolved early during Earth's history and has continued to evolve since then? In the following chapter we discuss the processes by which life evolved. In subsequent chapters, we will see how biologists determine the evolutionary histories of organisms, and how the millions of species that live today (as well as those that became extinct) formed from a single common ancestor. Finally, in Chapter 25, we will examine how life probably arose from nonliving matter several billion years ago.

Understanding biological evolution is important because evolutionary changes are taking place all around us. These

The Hands of Time

London's Big Ben, perhaps the most recognizable timepiece in the world, epitomizes our worldview of the importance of hours and minutes. Geological time, however, is much more difficult to grasp but is essential to understanding biological evolution.

changes have powerful implications for human welfare. Our own attempts to control populations of undesirable species and

increase populations of desirable ones make human beings powerful agents of evolutionary change. In addition to producing the results we desire, these efforts often cause undesirable outcomes, such as the evolution of resistance to medicines by pathogens and to pesticides by pests. Medicine and agriculture can respond creatively to the evolutionary changes they are causing only if their practitioners understand how and why those changes happen. But what exactly is biological evolution?

Biological evolution is a change over time in the genetic composition of a population. Changes that happen during the lifetimes of species constitute microevolution. Plant and animal breeding and changes occurring in response to environmental shifts over decades provide good examples of microevolution. Changes that involve the appearance of new species and evolutionary lineages are called macro-



380 CHAPTER TWENTY

evolution. The fossil record provides the best evidence of macroevolutionary changes among organisms. Many of these changes are dramatic.

To understand the long-term patterns of evolutionary change that we will document in this chapter, we must think in time frames spanning many millions of years and imagine events and conditions very different from those we now observe. The Earth of the distant past is, to us, a foreign planet inhabited by strange organisms. The continents were not where they are today, and climates were different. One of the remarkable achievements of modern science has been the development of sophisticated techniques for inferring past conditions and dating them accurately.

In this chapter, we first examine how events in the distant past can be dated. Then we review the major changes in physical conditions on Earth during the past 4 billion years, look at how those changes affected life, and discuss the major patterns in the evolution of life.

How Do We Know Earth Is Ancient?

It is difficult to age rocks because a rock of a particular type could have been formed at any time during Earth's history. It is easier to determine the ages of rocks relative to one another.

The first person to recognize that this could be done was the seventeenth-century Danish physician Nicolaus Steno. Steno realized that in an undisturbed sequence of sedimentary rocks, the oldest strata lie at the bottom and successively higher strata are progressively younger (Figure 20.1).

Geologists subsequently combined Steno's insights with their observations of the fossils—remains of ancient organisms—contained within rocks. They discovered that fossils of similar organisms were found in widely separated places on Earth, that certain organisms were always found in younger rocks than others, and that organisms in the most recent strata were more similar to modern organisms than those found in lower, more ancient strata. With this information, they were able to determine much about the relative ages of sedimentary rocks and about patterns in the evolution of life. But they still could not tell how old the rocks were. A method of dating rocks did not become available until the discovery of radioactivity at the turn of the twentieth century.

Radioactivity provides a way to date rocks

Radioactive isotopes decay in a regular pattern during successive, equal periods of time. During each successive time

RELATIVE TIME SPAN

~²⁰¹

Earth's Geological History

ERA

Cenozoic

PERIOD

Quaternary Tertiary

ONSET

1.8 mya 65 mya

MAJOR PHYSICAL CHANGES ON EARTH

:

Cold/dry climate; repeated glaciations Continents near current positions; climate cools

Cretaceous

Mesozoic Jurassic

Triassic

144 mya

206 mya 245 mya

Northern continents attached; Gondwana begins

to drift apart; meteorite strikes Yucatan

Peninsula Two large continents form: Laurasia (north) and

Gondwana (south); climate warm Pangaea slowly begins to drift apart; hot/humid

climate

c

.2

1

u

a

Paleozoic

Permian

Carboniferous

Devonian

Silurian

Ordovician

Cambrian

290 mya

354 mya 409 mya 440 mya 510 mya 543 mya

Continents aggregate into Pangaea; large

glaciers form; dry climates form in interior

of Pangaea Climate cools; marked latitudinal climate

gradients Continents collide at end of period; asteroid

probably collides with Earth Sea levels rise; two large continents form;

hot/humid climate Gondwana moves over South Pole; massive glaciation, sea level drops 50 m O_2 levels approach current levels

Precambrian

600 mya 2.5 bya 3.8 bya 4.5 bya

O_2 level at >5% of current level O_2 level at >1% of current level O_2 first appears in atmosphere



20.1 Young Rocks Lie on Top of Old Rocks

The oldest rocks at the bottom of this photo of the North Rim of the Grand Canyon formed about 540 million years ago. The youngest rocks on top are about 500 million years old.

MAJOR EVENTS IN THE HISTORY OF LIFE

Humans evolve; large mammals become extinct

Radiation of birds, mammals, flowering plants, and insects

Dinosaurs continue to radiate; flowering plants and

mammals diversify. Mass Extinction at end of period

(=76% of species disappear) Diverse dinosaurs; first birds; two minor extinctions Early dinosaurs; first mammals; marine invertebrates

diversify. Mass Extinction at end of period (=65% of species disappear)

Reptiles radiate; amphibians decline; Mass Extinction at end of period (=96% of species disappear)

Extensive "fern" forests; first reptiles; insects radiate; earliest flowering plants

Fishes diversify; first insects and amphibians. Mass Extinction at end of period (=75% of species disappear)

Jawless fishes diversify; first bony fishes; plants and animals colonize land

Mass Extinction at end of period (=75% of species disappear)

Most animal phyla present; diverse algae

Ediacaran fauna

Eukaryotes evolve; several animal phyla appear

Origin of life; prokaryotes flourish

THE HISTORY OF LIFE ON EARTH 381

interval, an equal fraction of the remaining radioactive material of any radioisotope decays, either changing to another element or becoming the stable isotope of the same element. For example, in 14.3 days, one-half of any sample of phosphorus-32 (^{32}P) decays to its stable isotope, phosphorus-31 (^{31}P). During the next 14.3 days, one-half of the remaining half decays, leaving one-fourth of the original ^{32}P . The time it takes for half of an isotope to decay is that isotope's half-life. After 42.9 days, three half-lives have passed, so one-eighth (that is, $V_1 \times V_2 \times V_2$) of the original ^{32}P remains.

Each radioisotope has a characteristic half-life. Which isotope is used to estimate the age of an ancient material depends on

how old the material is thought to be. Tritium (^3H) has a half-life of 12.3 years, and carbon-14 (^{14}C) has a half-life of about 5,700 years. The half-life of potassium-40 (^{40}K) is 1.3 billion years; the decay of potassium-40 to argon-40 has been used to date most of the ancient events in the evolution of life.

To use a radioisotope to date a past event, we must know or estimate the concentration of the isotope at the time of that event. In the case of carbon, we know that the production of new ^{14}C in the upper atmosphere (by the reaction of neutrons with ^{14}N) just balances the natural radioactive decay of ^{14}C . Therefore, the ratio of ^{14}C to its stable isotope, ^{12}C , exists in a more or less steady state in living organisms and their environment.

However, as soon as an organism dies, it ceases to exchange carbon compounds with the rest of the world. Its decaying ^{14}C is no longer replenished, and the ratio of ^{14}C to ^{12}C decreases. The ratio of ^{14}C to ^{12}C in fossil organisms can be used to date fossils (and thus the sedimentary rocks that contain those fossils) that are less than 50,000 years old with a fair degree of certainty.

Dating rocks more ancient than 50,000 years requires estimating isotope concentrations that exist in volcanic (but not in sedimentary) rocks. To date ancient sedimentary rocks, geologists search for places where volcanic ash or lava flows have intruded into beds of sedimentary rock. Radiometric dating, combined with observations of fossils, is the most powerful method of determining the ages of rocks.

But there are many places where sedimentary rocks do not contain suitable volcanic intrusions and few fossils are present. In these areas, dating methods other than radiometric must be used. One such method is based on the fact that Earth's magnetic poles move and occasionally reverse themselves. Because both sedimentary and igneous rocks preserve a record of Earth's magnetic field at the time they were formed, paleomagnetism helps determine the ages of those rocks. Other "time machines," which we will describe later, include continental drift, sea level changes, and molecular clocks.

Using these methods, geologists have divided Earth's history into eras, which in turn are subdivided into periods (Table 20.1). The boundaries between these divisions, which are based on major differences in the fossils con-

382 CHAPTER TWENTY

tained in successive layers of rocks, were established before the actual ages of the eras and periods were known.

Earth has undergone many physical changes that have influenced the evolution of life. The physical events we describe in this chapter, along with the most important milestones in the history of life, are listed in Table 20.1. Most of these biological milestones have taken place since the sudden explosion of new life forms that characterized the early Cambrian period, about 543 million years ago. The scale at the left of Table 20.1 gives a relative sense of geological time and the vast span of the Precambrian, during which early life evolved amid stupendous physical changes on Earth.

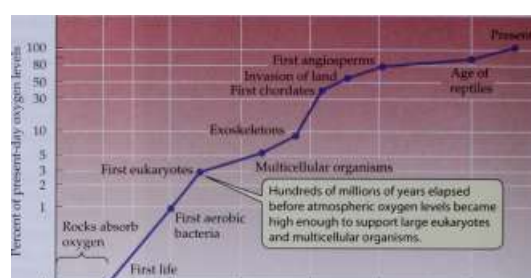
How Has Earth Changed over Time?

Two important physical changes on Earth have been unidirectional. First, Earth is cooling because it is continuously losing the heat that was generated when it formed. The radioactive furnace in Earth's core is steadily weakening, generating less and less heat to replace the heat that is lost to space. As a result, the processes that cause the continents to move about on Earth's surface have also weakened during Earth's history.

Second, Earth's atmosphere has changed unidirectionally. The atmosphere of early Earth probably had little or no free oxygen (O_2). Oxygen concentrations in the atmosphere began to increase markedly about 2.5 billion years ago, when certain sulfur bacteria evolved the ability to use water as a source of hydrogen ions for photosynthesis. The cyanobacteria that evolved from these sulfur bacteria liberated enough O_2 to open the way for the evolution of oxidation reactions as the energy source for ATP synthesis.

An atmosphere rich in oxygen also made possible larger cells and more complicated organisms. Small, unicellular aquatic organisms can obtain enough O_2 by simple diffusion even when O_2 concentrations are very low. Larger unicellular organisms have lower surface area-to-volume ratios (see Figure 4.2). In order to obtain enough O_2 by simple diffusion, they must live in an environment with a relatively high concentration of O_2 . Bacteria can thrive on 1 percent of the current atmospheric O_2 level, but eukaryotic cells require oxygen levels that are at least 2 to 3 percent of current atmospheric concentrations.

About 1,500 million years ago (mya), O_2 concentrations became high enough for large eukaryotic cells to flourish and diversify (Figure 20.2). Further increases in atmospheric O_2 levels 700 to 570 mya enabled multicellular organisms to evolve. The fact that it took many millions of years for Earth to develop an oxygenated atmosphere probably explains why only unicellular prokaryotes lived on Earth for more than a billion years.



Hundreds of millions of years elapsed before atmospheric oxygen levels became high enough to support large eukaryotes and multicellular organisms.

4,000 3,000 2,000

1,000 500

Millions of years ago

250

100

20.2 Large Cells Need More Oxygen

Although aerobic prokaryotes can flourish with less, large eukaryotic cells with lower surface area-to-volume ratios require at least 2 to 3 percent of current atmospheric O_2 concentrations. (Both axes of the graph are on logarithmic scales.)

Unlike the largely unidirectional changes in Earth's temperature and atmospheric O_2 concentrations, most physical changes on Earth have involved irregular oscillations in the planet's internal processes, such as volcanic activity and the shifting and colliding of continents. External events such as collisions with meteorites have also left their mark. In some cases, these events caused mass extinctions, wiping out a large proportion of the species living at the time.



The continents have changed position

The maps and globes that adorn our walls, shelves, and books give an impression of a static Earth. It would be easy for us to assume that the continents have always been where they are. But we would be wrong.

Earth's crust consists of solid plates approximately 40 km thick that float on a fluid mantle. The mantle fluid circulates because heat produced by radioactive decay in Earth's core sets up convection patterns. The plates move because material from the mantle rises and pushes them aside, resulting in seafloor spreading along ocean ridges. Where plates are pushed together, either they move sideways past each other, or one plate moves under the other, creating mountain ranges. The movement of the plates and the continents they contain—a process known as continental drift—has had enormous effects on climate, sea levels, and the distributions of organisms.

At times, the drifting of the plates has brought the continents together; at other times, they have drifted apart. The positions and sizes of the continents influence oceanic circulation patterns, sea levels, and global climate patterns. Mass extinctions of species, particularly marine organisms, have usually accompanied major drops in sea level (Figure 20.3).

High

Low



Asterisks indicate times of mass extinctions of marine organisms, most of which occurred when sea levels dropped.



Precambrian

Cambrian

Ordovician

Silurian

Devonian

Carboniferous

Permian

Triassic

Jurassic

Cretaceous

Tertiary



600

543

500

440

409

354 Millions of

290 245

years ago (mya)

206

144

' Quaternary 18

Present

20.3 Sea Levels Have Changed Repeatedly

Most mass extinctions of marine organisms have coincided with periods of low sea levels.

Earth's climate has shifted between hot/humid and cold/dry conditions

Through much of its history, Earth's climate was considerably warmer than it is today, and temperatures decreased more slowly toward the poles. At other times, however, Earth was colder than it is today. Large areas were covered with glaciers toward the end of the Precambrian and during the Carboniferous, Permian, and Quaternary periods, but these cold periods were separated by long periods of milder climates (Figure 20.4). Because we are living in one of the colder periods in the history of Earth, it is difficult for us to imagine the mild climates that were found at high latitudes during much of the history of life.

Usually climates change slowly, but major climatic shifts have taken place over periods as short as 5,000 to 10,000 years, primarily as a result of changes in Earth's orbit around the sun. A few climatic shifts appear to have been even more rapid. For example, during one Quaternary interglacial period, the Antarctic Ocean changed from being ice-covered to being nearly ice-free in less than 100 years.

Such rapid changes are usually caused by sudden shifts in ocean currents. Climates have sometimes changed so rapidly that extinctions caused by them appear "instantaneous" in the fossil record.

Volcanoes have disrupted evolution

On the morning of August 27, 1883, Krakatau, an island the size of Manhattan located in the Sunda Strait between Sumatra and Java, was devastated by a series of volcanic eruptions. Tidal waves caused by the eruption hit the shores of Java and Sumatra, demolishing towns and villages and killing 40,000 people. As impressive as this eruption was, however, its effects were local and short-lived. It did not cause major changes in patterns of the evolution of life. But much larger volcanic eruptions have occurred several times during Earth's history and have had major consequences for life.

During the late Permian period (about 275 mya), the continents came together to form a single gigantic land mass, Pangaea. This collision of continents caused massive

20.4 Hot/Humid and Cold/Dry Conditions Have Alternated over Earth's History

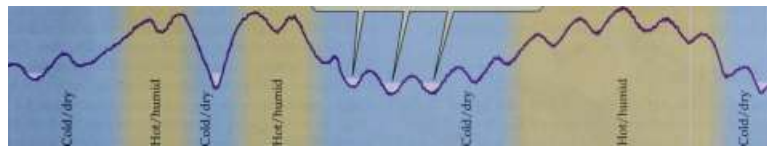
Throughout Earth's history, periods of cold climates and glaciations have been separated by long periods of milder climates.

High

—

Large areas of Earth's surface were covered by glaciers during these periods.

Low



Precambrian
 Cambrian
 Ordovician
 Silurian
 Devonian
 Carboniferous
 Permian
 Triassic
 Jurassic
 Cretaceous
 Tertiary



600 543 500 440 409 354 290 245

Millions of years ago (mya)

206

144

65 Quaternary 1

Present

384 CHAPTER TWENTY

volcanic eruptions. The ash the volcanoes ejected into Earth's atmosphere reduced the penetration of sunlight to Earth's surface, lowering temperatures, reducing photosynthesis, and triggering massive glaciation. Massive volcanic eruptions also occurred as the continents drifted apart during the late Triassic period and again at the end of the Cretaceous.

External events have triggered other changes on Earth

At least 30 meteorites between the sizes of baseballs and soccer balls hit Earth each year, but collisions with large meteorites are rare. In 1980, Luis Alvarez and several of his colleagues at the University of California, Berkeley, proposed that the mass extinction at the end of the Cretaceous period, about 65 mya, might have been caused by the collision of Earth with a large meteorite. These scientists based their hypothesis on the finding of abnormally high concentrations of the element iridium in a thin layer separating the rocks deposited during the Cretaceous period from those of the Tertiary (Figure 20.5). Iridium is abundant in some meteorites, but is exceedingly rare on Earth's surface.

To account for the estimated amount of iridium in this layer, Alvarez postulated that a meteorite 10 km in diameter collided with Earth at a speed of 72,000 km per hour. The force of such an impact would have ignited massive fires, created great tidal waves, and sent up an immense dust cloud that blocked the sun, thus cooling the planet. As it settled, the dust would have formed the iridium-rich layer.

This hypothesis generated a great deal of controversy and stimulated much research. Some scientists searched for the site of impact of the supposed meteorite. Others worked to improve the precision with which events of that age could be dated. Still others tried to determine more exactly the speed with which extinctions occurred at the Cretaceous-Tertiary boundary. Progress on all three fronts has favored the meteorite theory.

The theory was supported by the discovery of a circular crater 180 km in diameter buried beneath the northern coast of the Yucatan Peninsula of Mexico, thought to have been formed 65 mya. Recent fossil evidence also suggests that there may have been a sudden extinction of organisms 65 mya, as required by the meteorite theory. Therefore, most scientists accept that the collision of Earth with a large meteorite contributed importantly to the mass extinctions at the boundary between the Cretaceous and Tertiary periods.

The Fossil Record

Geological evidence is a major source of information about changes on Earth during the remote past. But the fossils preserved in the rocks—not the rocks themselves—are what have enabled geologists to order those events in time. What are fossils, and what do they tell us about the influence of physical events on the evolution of life on Earth? After examining the conditions that preserve the remains of organ-



20.5 Evidence of a Meteorite Collision with Earth

Iridium is a metal common in some meteorites, but rare on Earth. Its high concentrations in sediments deposited about 65 million years ago suggest the impact of a large meteorite.

isms, we will consider the completeness of the fossil record, and how that record reveals patterns in life's history.

An organism is most likely to become a fossil if its dead body is deposited in an environment that lacks oxygen. However, most organisms live in oxygenated environments and decompose when they die. Thus many fossil assemblages are collections of organisms that were transported by wind or water to sites that lacked oxygen. Occasionally, however, organisms, or imprints of them, are preserved where they lived. In such cases—especially if the environment in question was a cool, anaerobic swamp, where conditions for preservation were excellent—we can obtain a picture of communities of organisms that lived together.

How complete is the fossil record?

About 300,000 species of fossil organisms have been described, and the number is growing steadily. However, this number is only a tiny fraction of the species that have ever lived. We do not know how many species lived in the past, but we have ways of making reasonable estimates. Of the present-day biota—that is, all living species of all kinds—approximately 1.6 million species have been named. The actual number of living species is probably at least 10 million. It is possibly higher than 50 million, because most species of insects (the animal group with the largest number of species; see Chapter 32) have not yet been described. So the number of known fossil species is less than 2 percent of the probable minimum number of living species.

Because life has existed on Earth for about 3.8 billion years, and because most species exist, on average, for fewer than 10 million years, the species living on Earth must have turned over many times during geological history, and the total number of species that have lived over evolutionary time must vastly exceed the number living today

The number of known fossils, although small in relation to the total number of extinct species, is higher for some



20.6 A Fossil Spider

Trapped in sap of a tree in what is now Arkansas about 50 mya, this spider is exquisitely preserved in the amber formed from the sap. The details of its external anatomy are clearly visible.

groups of organisms than for others. The record is especially good for marine animals that had hard skeletons. Among the nine major animal groups with hard-shelled members, approximately 200,000 species have been described from fossils, roughly twice the number of living marine species in these same groups. Paleontologists lean heavily on these groups in their interpretations of the evolution of life in the past. Insects and spiders are also relatively well represented in the fossil record (Figure 20.6).

The fossil record demonstrates several patterns

Despite its incompleteness, the fossil record reveals several patterns that are unlikely to be altered by future discoveries. First, great regularity exists. For example, organisms of particular types are found in rocks of specific ages, and new organisms appear sequentially in younger rocks. Second, as we move from ancient periods of geological time toward the

present, fossil species increasingly resemble species living today. The fossil record also tells us that extinction is the eventual fate of all species.

The fossil record contains many series of fossils that demonstrate gradual change in lineages of organisms over time. A good example is the series of fossils showing the pathway by which whales evolved from hoofed terrestrial mammals, beginning about 50 mya. Fossils that are intermediate between whales and their terrestrial ancestors illustrate the major changes by which whales became adapted for aquatic existence and lost their hind limbs (Figure 20.7).

Interestingly, whales retain the genetic potential for developing legs; occasionally, living whales have been found with small hind legs that extend outside their bodies. The claim (made repeatedly by scientific creationists) that the fossil record does not contain examples of such intermediates is false. Intermediates abound, and more and more of them are being discovered.

But the incompleteness of the fossil record can mislead us when we try to interpret it. Organisms may have evolved in places where their fossils have not been discovered. Moreover, when a species that evolved in one place appears among the fossils at another site, it gives the false impression that it evolved very rapidly from one of the species that already lived there.

Horses, for example, evolved at varying rates over millions of years in North America. Many different lineages arose and died out (Figure 20.8). Ancestors of horses crossed the Bering land bridge into Asia at several different times, the most recent one only several million years ago. Evidence of each crossing appears suddenly in the Asian fossil record as a major new type of horse. If we lacked fossil evidence of horse evolution in North America, we might conclude that horses evolved very rapidly somewhere in Asia. On the other hand, an incomplete fossil record can also hide rapid changes.

By combining data about physical events during Earth's history with evidence from the fossil record, scientists can compose pictures of what Earth and its inhabitants looked like at different times. We know in general where the continents were and how life changed over time, but many of the details are poorly known, especially for events in the more remote past. In the next section we provide an overview of the major patterns in the history of life on Earth.

Cambrian Ordovician Silurian Devonian [Carboniferous Permian

Triassic

Jurassic

543 5 440 409 354 2 L »0 245 206

Millions of years ago (mya)

20.7 From Terrestrial to Aquatic Life

An artist's reconstruction, based on fossil skeletons, of four ancestors representing different stages in the evolution of modern whales from an early terrestrial mammal.



Mesonychid 55 mya

Ambulocetus

52 mya

Rodhocetus

46 mva

Basilosaurus 42 mya

543 500 440 409 354 290 245 206

Millions of years ago (mya)

Early Tertiary (50 mya)

t

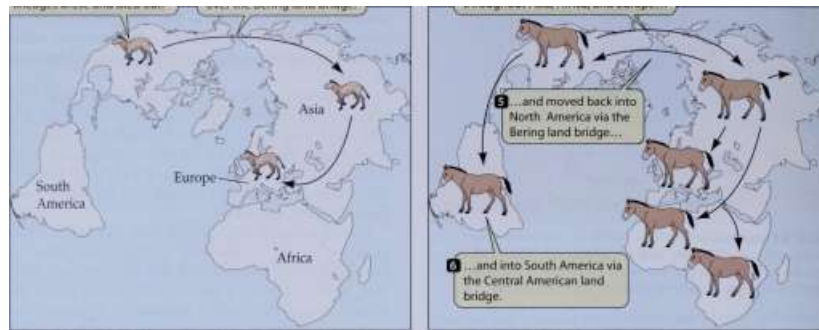
The earliest horses evolved in North America, where many lineages arose and died out.



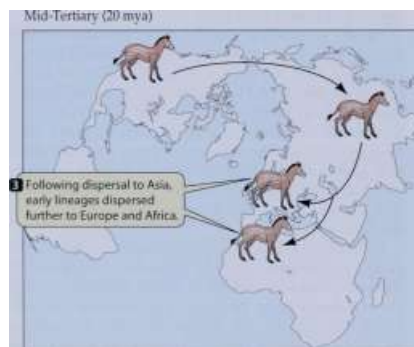
o Ancestors of several of these lineages crossed into Asia over the Bering land bridge.

Quaternary (4 mya)

EJ Ancestors of Equus, the modern horse, dispersed from North America throughout Asia, Africa, and Europe...



Mid-Tertiary (20 mya)



20.8 Horses Have a Complex Evolutionary History

Ancestors of horses crossed the Bering land bridge into Asia several times, the last one only a few million years ago. If we lacked the earlier fossil evidence of horse evolution in North America, we might reach the false conclusion that horses evolved rapidly somewhere in Asia.

Life in the Remote Past

Life first evolved about 3.8 billion years ago (bya). The major groups of eukaryotic organisms evolved during the Precambrian, about 2.5 bya. The fossil record of organisms that lived prior to the Cambrian period is fragmentary, but shows that the volume of organisms increased dramatically in late Precambrian times, about 650 mya (see Table 20.1). The shallow Precambrian seas teemed with life. Protists and small multicellular animals fed on floating algae. Living plankton and plankton remains were devoured by animals that filtered food from the water or ingested sediments and digested the organic material in them.



20.9 Ediacaran Animals

These fossils of soft-bodied invertebrates, excavated at Ediacara in southern Australia, formed 600 million years ago. They illustrate the diversity of life that evolved in Precambrian times.

The best fossil assemblage of Precambrian animals, all soft-bodied invertebrates, was discovered at Ediacara, in southern Australia (Figure 20.9). The Ediacaran fauna is very different from any assemblage of animals living today. Some of its members may represent animal lineages that have no living descendants.

Diversity exploded during the Cambrian

By the early Cambrian period (543-510 mya), oxygen levels in Earth's atmosphere approached their current concentrations, and the continental plates came together in several masses, the largest of which was Gondwana (Figure 20.10). The three great evolutionary lineages of animals separated and began to radiate during this period. Evolutionary radiation—the proliferation of species within a single lineage—during this time resulted in the dramatic increase in diversity known as the Cambrian explosion. All of the major groups of animals that have species living today appeared during the Cambrian, as did animals belonging to many lineages that have left no surviving descendants.

The most extensive fossil evidence from the Cambrian period comes from an unusually well preserved fauna recently discovered in China (Figure 20.10b)- Arthropods are the most diverse group in the Chinese fauna; some of them were large carnivores. A mass extinction occurred at the end of the Cambrian.

Major changes continued during the Paleozoic era

Because they have excellent fossil evidence and can date events relatively precisely, geologists have divided the remainder of the Paleozoic era into five periods: the Ordovician, Silurian, Devonian, Carboniferous, and Permian periods (see Table 20.1).

(«)

Cambrian Ordovician Silurian Devonian Carboniferous Permian

Triassic

Jurassic

Cretaceous

440 409 354 290 245 206 144

Millions of years ago (mya)

North Pole

The view of Earth has been distorted here so that you can see both poles.



the ordovician (510-440 mya). During the Ordovician period, the continents were located primarily in the Southern Hemisphere. Evolutionary radiation of marine organisms was spectacular during the early Ordovician, especially among animals (such as brachiopods and mollusks) that filter small prey from the water. All animals lived on the seafloor or burrowed in its sediments. Ancestors of club mosses and horsetails colonized wet terrestrial environments, but they were still relatively small. At the end of the Ordovician, sea levels dropped about 50 meters as massive glaciers formed over Gondwana, and ocean temperatures dropped. About 75 percent of the marine animal species became extinct, probably because of these major environmental changes.

the Silurian (440-409 mya). During the Silurian period, the northern continents coalesced, but the general positions of the continents did not change much. Marine life rebounded from the mass extinction at the end of the Ordovician. Animals able to swim and feed above the ocean bottom appeared for the first time, but no major new groups of marine organisms evolved. The tropical sea was uninterrupted by land barriers, and most marine genera were widely distributed. On land, the first terrestrial arthropods—scorpions and millipedes—appeared.

the devonian (409-354 mya). Rates of evolutionary change accelerated in many groups of organisms during the Devonian period. Northern and southern land masses slowly moved northward (Figure 20.11 ^). There was a great evolutionary radiation of corals and shelled squidlike cephalopods (Figure 20.11fr). Fishes diversified as jawed forms replaced jawless ones, and heavy armor gave way to the less rigid outer coverings of modern fishes. All current major groups of fishes were present by the end of the period.

Terrestrial communities also changed dramatically during the Devonian. Club mosses, horsetails, and tree ferns became common, and some reached the size of trees. Their deep roots accelerated the weathering of rocks, resulting in the development of the first forest soils. Distinct floras

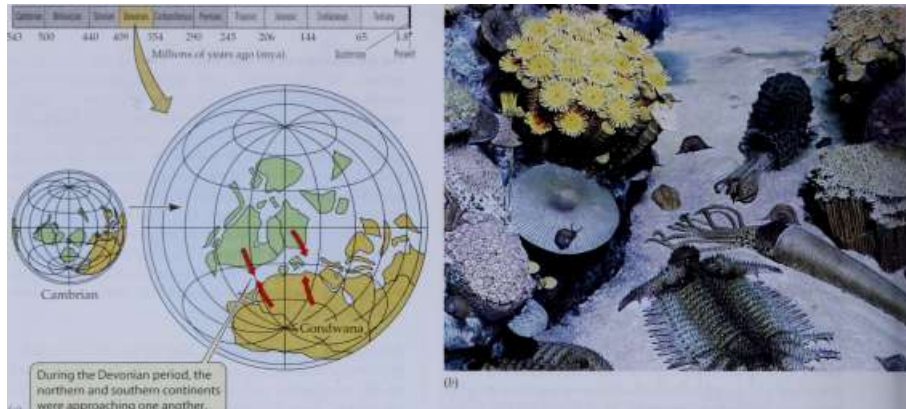


South Pole

This group of land masses is gradually moving together to form Gondwana.

20.10 Cambrian Continents and Animals

(a) Positions of the continents during mid-Cambrian times (543-510 mya). (b) Fossil beds in China have yielded excellent remains of Cambrian animals including *Jianfangia*, a predatory arthropod.



(a)

During the Devonian period, the northern and southern continents were approaching one another.

20.11 Devonian Continents and Marine Communities

(a) Positions of the continents during the Devonian period (409-354 mya). (b) This artist's reconstruction shows how a Devonian reef may have appeared.

evolved on the two land masses toward the end of the period, and the first gymnosperms appeared. The first known fossils of centipedes, spiders, pseudoscorpions, mites, and insects date to this period, and fishlike amphibians began to occupy the land.

An extinction of about 75 percent of all marine species marked the end of the Devonian. Paleontologists disagree on the cause of this mass extinction. Some believe that it was triggered by the collision of the two continents, which destroyed much of the existing shallow, warm-water marine environment. This hypothesis is supported by the fact that extinction rates were much higher among tropical than among cold-water species.

the carboniferous (354-290 mya). Large glaciers formed over high-latitude Gondwana during the Carboniferous period, but extensive swamp forests grew on the tropical continents. These forests were not made up of the kinds of trees we know today, but were dominated by giant tree ferns and horsetails (see Figure 28.9). Fossilized remains of those "trees" formed the coal that we now mine for energy.

The diversity of terrestrial animals increased greatly. Snails, scorpions, centipedes,

20.12 A Carboniferous "Crinoid Meadow"

Crinoids, which were dominant marine animals during the Carboniferous (354-290 mya), may have formed communities that looked like this. Sharks and bony fishes were important members of these communities.

and insects were abundant and diverse. Insects evolved wings, which gave them access to tall plants; plant fossils from this period show evidence of insect damage. Amphibians became larger and better adapted to terrestrial existence. From one amphibian stock, the first reptiles evolved late in the period. In the seas, crinoids reached their greatest diversity, forming meadows on the seafloor (Figure 20.12).

the permian (290-245 mya). During the Permian period, the continents coalesced into a supercontinent—Pangaea. Massive volcanic eruptions resulted in outpourings of lava that covered large areas of Earth (Figure 20.13). The ash they produced blocked the sunlight, cooling the climate and resulting in the largest glaciers in Earth's history.

Permian deposits contain representatives of most modern groups of insects. By the end of the period, reptiles greatly outnumbered amphibians. Late in the period, the lineage leading to mammals diverged from one reptilian lineage. In fresh waters, the Permian period was a time of extensive radiation of bony fishes.

543 500 440 409 354 290 245 206 144

Millions of years ago (mya)



Mria

Mmim

Baa

Dmm

Carteni'eiws

Penman ' Tnassc

500

440 409

Jurass:

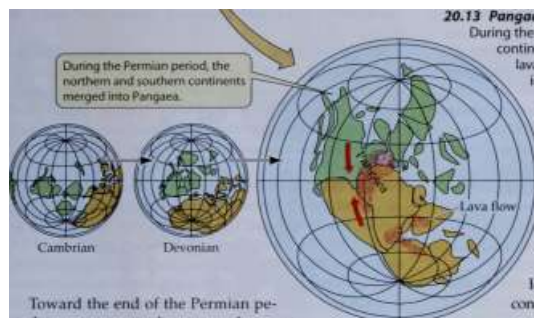
Cretaceous

354 2Vo ^\245 206 144

Millions of years ago (mya)



During the Permian period, the northern and southern continents merged into Pangaea.



Cambrian

Toward the end of the Permian period, two events may have caused separate mass extinctions. The first event was the massive outpouring of volcanic lava, which drastically reduced the oxygen content of deep ocean waters. The second was a rapid turnover of the oceans that brought oxygen-depleted deep waters to the surface. These waters released toxic concentrations of carbon dioxide and hydrogen sulfide into surface waters and the atmosphere, poisoning most species.

Geographic differentiation increased during the Mesozoic era

At the start of the Mesozoic era (250 mya), the few surviving organisms found themselves in a relatively empty world. As Pangaea slowly separated into individual continents, the glaciers melted, and the oceans rose and re-flooded the continental shelves, forming huge, shallow inland seas. Life again proliferated and diversified, but different lineages came to dominate Earth. The large plants that dominated the great coal-forming forests, for example, were replaced by new plant lineages in which seeds had evolved.

THE HISTORY OF LIFE ON EARTH 389

20.13 Pangaea Formed in the Permian Period

During the Permian (290-245 mya), the interior of the "super-continent" Pangaea experienced harsh climates. Massive lava flows spread over Earth, and the largest glaciers in Earth's history formed during this period.

During the Mesozoic, Earth's biota, which until that time had been relatively homogeneous, became increasingly provincialized. Distinct terrestrial floras and faunas evolved on each continent. The biotas of the shallow waters bordering the continents also diverged from one another. The localization that began during the Mesozoic continues to influence the geography of life today.

the triassic (245-206 mya). During the Triassic period, many invertebrate lineages became more diverse, and many burrowing forms evolved from groups living on the surfaces of bottom sediments. On land, conifers and seed ferns became the dominant trees. The first frogs and turtles appeared. A great radiation of reptiles began, which eventually gave rise to dinosaurs, crocodilians, and birds. The end of the Triassic was marked by a mass extinction that eliminated about 65 percent of species on Earth. Why they went extinct is not known, but a meteor impact is suspected.

the Jurassic (206-144 mya). The mass extinction at the close of the Triassic was followed by another period of evolutionary diversification during the Jurassic period. Bony fishes began the great radiation that culminated in their dominance of the oceans. Salamanders and lizards first appeared. Flying reptiles evolved, and dinosaur lineages evolved into bipedal predators and large quadrupedal herbivores (Figure 20.14). Several groups of mammals first appeared during this time.

Carabnan

Mm h

9ra

Ikjh

ii

Carboniferous Permian Triassic

Jurassic

Cret2c«us

Tertiary

543 500 440 409 354 290 245 206 144

Millions of years ago (mya)

20.74 Mesozoic Dinosaurs

The dinosaurs of the Mesozoic era continue to capture our imagination. This painting illustrates some of the large species from the Jurassic period (206-144 mya).



390 CHAPTER TWENTY

the cretaceous (144-65 mya). By the early Cretaceous period, the northern continents were completely separate from the southern ones, and a continuous sea encircled the Tropics (Figure 20.15). Sea levels were high, and Earth was warm and humid. Life proliferated both on land and in the oceans. Marine invertebrates increased in variety and number of species. On land, dinosaurs continued to diversify. The first snakes appeared during the Cretaceous, though their lineage did not radiate until much later.

Early in the Cretaceous, flowering plants—the angio-sperms—evolved from gymnosperm ancestors and began the radiation that led to their current dominance on land. By the end of the period, many groups of mammals had evolved, but these mammals were generally small.

Another mass extinction took place at the end of the Cretaceous period. On land, all vertebrates larger than about 25 kg in body weight apparently became extinct. In the seas, many planktonic organisms and bottom-dwelling invertebrates became extinct. This mass extinction was probably caused by the large meteorite that collided with Earth off the Yucatan Peninsula, as described on page 384.

The modern biota evolved during the Cenozoic era

By the early Cenozoic era (65 mya), the positions of the continents resembled those of today, but Australia was still attached

to Antarctica, the Atlantic Ocean was much narrower, and the northern continents were connected. The Cenozoic era was characterized by an extensive radiation of mammals, but other groups were also undergoing important changes. Flowering plants diversified extensively and dominated world forests, except in cool regions.

the tertiary (65-1.8 mya). During the Tertiary period, Australia began its northward drift. By 20 mya it had nearly reached its current position. The map of the world during this period looks familiar to us. In the middle of the Tertiary, the climate became considerably drier and cooler. Many lineages of flowering plants evolved herbaceous

(nonwoody) forms, and grasslands spread over much of Earth.

By the beginning of the Cenozoic era, invertebrate faunas resembled those of today. It is among the vertebrates that evolutionary changes during the Tertiary were most rapid. Living groups of reptiles, including snakes and lizards, underwent extensive radiations during this period, as did birds and mammals.

the quaternary (1.8 mya-present). The current geological period, the Quaternary period, is subdivided into two epochs, the Pleistocene and the Holocene (also known as the Recent). The Pleistocene epoch, which began about 1.8 mya, was a time of drastic cooling and climatic fluctuations. During four major and about twenty minor episodes, massive glaciers spread across the continents. Earth became much cooler, and animal and plant populations shifted toward the equator. The last of these glaciers retreated from temperate latitudes less than 15,000 years ago. Organisms of the current Holocene epoch are still adjusting to these changes; many high-latitude ecological communities have occupied their current locations for no more than a few thousand years.

Interestingly, these climate fluctuations resulted in few extinctions. However, the Pleistocene was the scene of hominid evolution and radiation, resulting in the species *Homo sapiens* —modern humans (see Chapter 33). Many large birds and mammals became extinct in North and South America and in Australia when *H. sapiens* arrived on those continents. Human hunting may have caused these extinctions, although the existing evidence does not convince all paleontologists.

Rates of Evolutionary Change

Following each mass extinction, the diversity of life rebounded. How fast did evolution proceed during those times? Why did some lineages evolve rapidly while others

Cambrian Oriorician Silurian Devonian Carboniferous Permian

Tnassic

Jurassic

Cretaceous

Tertiary

543 500

440 409

354 290 245 206 144

Millions of years ago (mya)

Quaternary

You can begin to make out the forms of what will become North America, South America, and Africa.



Cambrian

Devonian

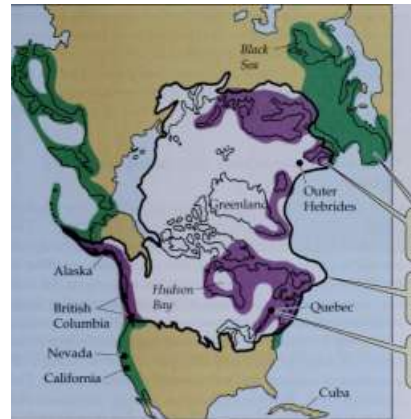
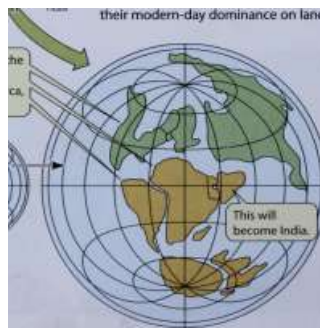
Permian

, L8 I

Present

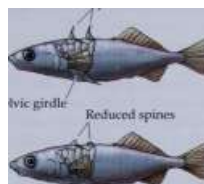
20.15 Continents during the Cretaceous Period

Many lineages of small mammals evolved during the Cretaceous (144-65 mya), and flowering plants began the radiation leading to their modern-day dominance on land.



Spines

Nevada California



Pelvic girdle

20.16 Natural Selection Acts on Stickleback Spines

Three-spined stickleback populations with reduced spines are found principally in young lakes that were covered by ice during the most recent glacial period. These lakes lack large predatory fishes, but contain predatory insects that capture the fish by grasping their spines.

The current range of sticklebacks includes formerly glaciated areas (lavender) and unglaciated areas (green).

did not? Scientists have made enough progress in studying evolution to be able to give at least tentative answers to these questions.

Evolutionary rates vary

The fossil record shows that rates of evolution have been uneven. Many species have experienced times of stasis, long periods during which they changed very little. For example, many marine lineages have evolved slowly. Horseshoe crabs that lived 300 mya are almost identical in appearance to those living today, and the chambered nautilus of the late Cretaceous are indistinguishable from living species. Such "living fossils" are found today in harsh environments that have changed relatively little for millennia. The sandy coastlines where horseshoe crabs spawn have extremes in temperature and salt concentration that are lethal to many other organisms. Chambered nautilus spend their days in deep, dark ocean waters, ascending to feed in food-rich surface waters only under the protective cover of darkness. Their intricate shells provide little protection against today's visually hunting fishes.

Periods of stasis may be broken by times during which changes, either in the physical or the biological environment, create conditions that favor new traits. How new conditions favor rapid evolutionary change is illustrated by the spines of the three-spined stickleback (*Gasterosteus aculeatus*). This widespread marine fish has repeatedly invaded fresh water throughout its evolutionary history (Figure 20.16).

Sticklebacks are tiny fish, usually less than 10 cm long. All marine and most freshwater populations have well-developed pelvic girdles with prominent spines that make it difficult for other fishes to swallow them. However, large

The region of the Northern Hemisphere that was once covered by Pleistocene glaciers is outlined in black.

Places where sticklebacks are known to have reduced spines are indicated by circles.

predatory insects can readily grasp the stickleback's spines, and prey selectively on stickleback individuals with the largest spines. When stickleback populations invade freshwater habitats where predatory fish are absent but predatory insects are present, they rapidly evolve smaller spines. Populations with reduced spines are found primarily in young lakes that were covered by ice during the most recent glaciation, and hence do not have large predatory fishes.

The extensive fossil record of sticklebacks shows that spine reduction evolved many times in different populations that invaded fresh water. In addition, molecular data reveal that each freshwater population is most closely related to an adjacent marine population, not to other freshwater populations. Therefore, spine reduction has evolved rapidly many times in different places in response to the same ecological situation: the absence of predatory fish.

Extinction rates vary over time

More than 99 percent of the species that have ever lived are extinct. Species have become extinct throughout the history of life, but extinction rates have fluctuated dramatically over time; some groups had high extinction rates while others were proliferating.

Each mass extinction changed the flora and fauna of the next period by selectively eliminating some types of organisms, thereby increasing the relative abundance of others. For example, among the seashells of the Atlantic coastal plain of North America, species with broad geographic ranges were less likely to become extinct during normal periods (when no mass extinctions were taking place) than were species with small geographic ranges.

On the other hand, during the mass extinction of the late Cretaceous, groups of closely related species with large geographic ranges survived better than groups with small ranges, even if the individual species within the group had small ranges. Similar patterns are found in other molluscan groups elsewhere, suggesting that traits favoring long-term

392 CHAPTER TWENTY

survival during normal times are often different from those that favor survival during times of mass extinctions.

At the end of the Cretaceous period, extinction rates on land were much higher among large vertebrates than among small ones. The same was true during the Pleistocene mass extinction, when extinction rates were high only among large mammals and large birds. During some mass extinctions, marine organisms were heavily hit while terrestrial organisms survived well. Other extinctions affected organisms living in both environments. These differences are not surprising, given that major changes on land and in the oceans did not always coincide.

Patterns of Evolutionary Change

Major new features, such as the feathers of birds or the legs of terrestrial vertebrates, that adapt organisms to a special way of life are called evolutionary innovations. How such novelties arise has been the subject of much debate from Darwin's time to the present. The variety of sizes and shapes among living organisms seems almost limitless, but the number of truly novel structures is remarkably small. As fiction writers often do, we can imagine unusual vertebrates with wings sprouting from their backs, but in reality the wings of vertebrates are always modified front legs. Modern mammals are highly varied in their shapes, but all

20.17 Evolutionary Faunas

Representatives of the three major evolutionary faunas are shown together with a graphic illustration of the number of families in each fauna over time.

of their structures are modifications of structures found in ancestral mammals. As we saw earlier, even transforming a terrestrial mammal into a whale did not require a drastic reorganization of the mammalian body plan. Only a few evolutionary innovations, such as the notochord of chordates, do not appear to be modifications of a preexisting structure.

Three major faunas have dominated animal life on Earth

Only three events during the evolution of life have resulted in the evolution of major new faunas (Figure 20.17). The first one, the Cambrian explosion, took place about 540 mya. The second, about 60 million years later, resulted in the Paleozoic fauna. The great Permian extinctions 300 million years later were followed by the third event, the Triassic explosion, which led to our modern fauna.

During the Cambrian explosion, organisms representative of all major present-day lineages appeared, along with a number of lineages that subsequently became extinct. The Paleozoic and Triassic explosions greatly increased the number of families, genera, and species, but no new or dramatically different organismal body plans evolved. The later explosions resulted in many new organisms, but all of them were modifications of body plans that were already present when these great biological diversifications began.

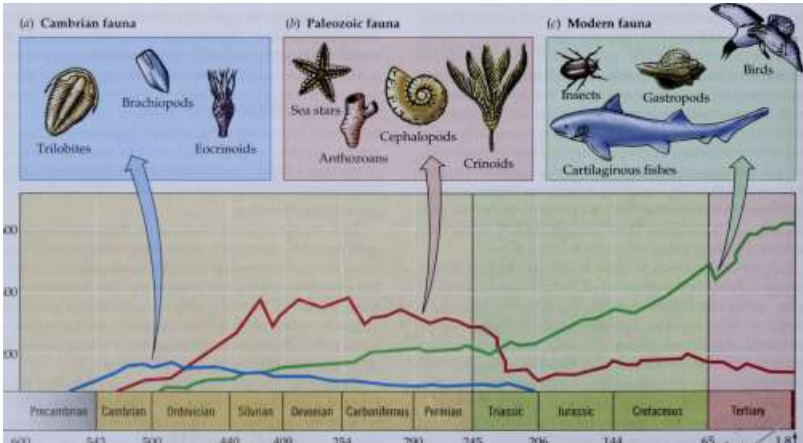
Biologists have long puzzled over the striking differences between the Cambrian explosion and the two later explosions. A commonly accepted theory is that because the Cambrian explosion took place in a world that con-

(a) Cambrian fauna

(b) Paleozoic fauna

~0 400

1
3



Quaternary

Present

20.18 Snail Shells Have Thickened over Time

The percentage of families of snails that have internally thickened or narrowed openings to their shells has increased with evolutionary time—evidence that predation on shelled animals intensified.

tained only a few species of organisms, all of which were small, the ecological setting was favorable for the evolution of many new body plans and different ways of life. Many types of organisms were able to survive initially in this world, but as competition intensified and new types of predators evolved, many forms were unable to persist.

Although Earth was relatively poor in species at the time of the two later explosions as well, the species that were already present included a wide array of body plans and ways of life. As body plans became more specialized, major transformations of form became increasingly less likely. Therefore, major new innovations were less likely to evolve at these times than in the Cambrian.

The size and complexity of organisms have increased

The earliest organisms were small prokaryotes. A modest increase in size and a dramatic increase in structural complexity accompanied the evolution of the first eukaryotes 2.5 billion years ago. Since then, the maximum sizes of organisms in many lineages have increased, irregularly to be sure. The most striking exception to this trend is insects, which have remained relatively small throughout their evolutionary history.

The overall increase in body size is the result of two opposing forces. Within a species, selection often favors larger size because larger individuals can dominate smaller ones. But larger species on average survive for less time than small species do, which is one reason why Earth is not populated primarily by large organisms.

Predators have become more efficient

Over time, predators have evolved increasingly efficient methods of capturing prey, and prey, in turn, have evolved better defenses. During the Cretaceous, for example, many

species of crabs with powerful claws evolved, and carnivorous marine snails able to drill holes in shells began to fill the seas. Skates, rays, and bony fishes with powerful teeth capable of crushing mollusk shells also evolved, and large, powerful marine reptiles—the placodonts—fed heavily on clams. The increasing thickness and narrowing openings of snail shells during the Cretaceous is evidence that predation rates intensified (Figure 20.18). Other evidence of heavy predation pressure is the increase in the percentage of fossil shells that show signs of having been repaired following an attack that did not kill the owner.

Although shell thickness provided some protection from predators, predators were so effective that clams disappeared from the surfaces of most marine sediments. The survivors were species that burrowed into the substratum, where they were more difficult to capture.

The Future of Evolution

The agents of evolution are operating today just as they have been since life first appeared on Earth. However, major changes are under way as a result of the dramatic increase of Earth's human population. Until recently, human-caused extinctions affected mostly large vertebrates, but these losses are now being compounded by increasing extinctions of small species, driven primarily by changes in Earth's vegetation. Deliberately or inadvertently, people are moving thousands of species around the globe, reversing the provincialization of Earth's biota that evolved during the Mesozoic era.

Humans have also taken charge of the evolution of certain valuable species by means of artificial selection and biotechnology. Our ability to modify species has been enhanced by modern molecular methods that enable us to move genes among species—even distantly related ones. In short, humans have become the dominant evolutionary agent on Earth today. How we handle our massive influence will powerfully affect the future of life on Earth.

394 CHAPTER TWENTY

Chapter Summary

► Changes that take effect during the lifetimes of species constitute microevolution. Changes that involve the appearance of new species and evolutionary lineages are called macroevolution.

How Do We Know that Earth is Ancient?

- ▶ The relative ages of rock layers in Earth's crust can be determined from their positions relative to one another and from their embedded fossils.
- ▶ Radioisotopes supplied the key for assigning absolute ages to rocks.
- ▶ Earth's geological history is divided into eras and periods. The boundaries between these units are based on differences between their fossil biotas. Review Table 20.1

How Has Earth Changed over Time?

- ▶ Unidirectional physical changes on Earth include gradual cooling and weakening of the forces that cause continental drift.
- ▶ Earth's early atmosphere lacked free oxygen. Oxygen accumulated after prokaryotes evolved the ability to use water as their source of hydrogen ions in photosynthesis. Increasing concentrations of atmospheric oxygen made possible the evolution of eukaryotes and multicellular organisms. Review Figure 20.2
- ▶ Throughout Earth's history the continents have moved about, sometimes separating from one another, at other times colliding. Review Figures 20.10, 20.11, 20.13, 20.15
- ▶ Earth has experienced periods of rapid climate change, massive volcanism, and major shifts in sea levels and ocean currents, all of which have had dramatic effects on the evolution of life. Review Figures 20.3, 20.4
- ▶ External events, such as collisions with meteorites, also have changed conditions on Earth. A meteorite may have caused the abrupt mass extinction at the end of the Cretaceous period.

The Fossil Record

- ▶ Much of what we know about the history of life on Earth comes from the study of fossils.
- ▶ The fossil record, although incomplete, reveals broad patterns in the evolution of life. About 300,000 fossil species have been described. The best record is that of hard-shelled marine animals.
- ▶ Fossils show that many evolutionary changes are gradual, but an incomplete record can falsely suggest or conceal times of rapid change. Review Figures 20.7, 20.8

Life in the Remote Past

- ▶ The fossil record for Precambrian times is fragmentary, but fossils from Australia show that many lineages that evolved then may not have left living descendants.
- ▶ Diversity exploded during the Cambrian period. Review Figure 20.10
- ▶ Geographic differentiation of biotas increased during the Mesozoic era.
- ▶ The modern biota evolved during the Cenozoic era.

Rates of Evolutionary Change

- ▶ Rates of evolutionary change have been very uneven.
- ▶ Rapid rates of evolution occur when changes to the physical or biological environment create conditions that favor new traits. Review Figure 20.16

Patterns of Evolutionary Change

- ▶ Truly novel features of organisms have evolved infrequently. Most evolutionary changes are the result of modifications of already existing structures.
- ▶ Three major faunas have dominated animal life on Earth. Review Figure 20.17
- ▶ Over evolutionary time, organisms have increased in size and complexity. Predation rates have also increased, resulting in the evolution of better defenses among prey species. Review Figure 20.18

The Future of Evolution

- ▶ The agents of evolution continue to operate today, but human intervention, both deliberate and inadvertent, now plays an unprecedented role in the history of life.

For Discussion

1. Some lineages of organisms have evolved to contain large numbers of species, whereas others have produced only a few species. Is it meaningful to consider the former more successful than the latter? What does the word "success" mean in evolution? How does your answer influence your thinking about *Homo sapiens*, the only surviving representative of the

Hominidae—a family that never had many species in it?

2. Scientists date ancient events using a variety of methods, but nobody was present to witness or record those events. Accepting those dates requires us to believe in the accuracy and appropriateness of indirect measurement techniques. What other basic scientific concepts are based on the results of indirect measurement techniques?
3. Why is it useful to be able to date past events absolutely as well as relatively?
4. What factors favor increases in body size? Why might average body size among particular species in a lineage decrease even if natural selection favors larger body size in most species of that lineage?
5. The continents are still drifting today, but biologists ignore these movements when thinking about factors affecting current evolutionary changes. On what basis do they make that decision?



The Mechanisms of Evolution

Most species of cuckoos and cowbirds lay their eggs in the nests of other species of birds. This behavior is known as brood parasitism. The host birds often incubate the eggs and raise the parasite nestlings. Host birds that accept parasite eggs and raise parasite chicks are likely to produce fewer offspring than hosts that recognize parasite eggs and push them out of the nest.

To investigate the evolution of such defensive behaviors, biologists studied cuckoos and their hosts in areas where brood parasitism had been occurring for different periods of time. In one valley in southern Spain, great spotted cuckoos and common magpies have lived together for many centuries. Here 78 percent of the magpies removed artificial cuckoo eggs experimenters placed in their nests. However, in another Spanish valley, where cuckoos did not arrive until the early 1960s, only 14 percent of magpies ejected the eggs.

Ejection of parasites' eggs evolved rapidly in Japan, where the ranges of the common cuckoo and the azure-winged magpie have only recently overlapped. In a region where cuckoos have parasitized magpies for 10 years, none of the magpies ejected cuckoo eggs, but in areas where they have been parasitized for 20 years, 42 percent of the magpies ejected cuckoo eggs.

What explains these differences in magpie behavior? Charles Darwin's main contribution to biology was to propose a plausible and testable hypothesis of a mechanism of evolutionary change that could result in the adaptation of organisms to their environments. Keep in mind that the fitness of an organism includes the physical environment, individuals of other species, and individuals of the same species. All of these components influence the survival and reproductive success of individuals.

In this chapter we will review how Darwin developed his ideas, and then turn to the advances in understanding of evolutionary processes since Darwin's time. We will discuss the genetic basis of evolution and show how genetic variation within populations is measured. We will describe the agents of evolution and show how biologists design studies to investigate them. Finally, we will discuss con-

A Magpie and a Cuckoo Chick

In parts of Japan, the azure-winged magpie has only recently experienced brood parasitism by the common cuckoo. This adult magpie will care for the cuckoo chick at the expense of its own offspring.

straints on the pathways evolution can take. When you understand these processes, you will understand the mechanisms of evolution.

Charles Darwin and Adaptation

The term adaptation has two meanings in evolutionary biology. The first refers to the traits that enhance the survival and reproductive success of their bearers. For example, we believe that wings are adaptations for flight, a spider's web is an adaptation for capturing flying insects, and so forth. The second refers to the means by which these traits are acquired—that is, the processes that produce them.

Biologists regard an organism as being adapted to a particular environment when they can imagine—or better still, measure the performance of—a slightly different organism that reproduces and survives less well in that environment. That is, adaptation is a relative concept; to understand adaptation, biologists compare the performance of individuals that differ in traits within and among species. For example, to investigate the adaptive nature of spiders' webs, we would try to determine the effectiveness of slightly different webs spun by a given species in capturing insects. We would also measure changes in the webs of the species in different situations. With these data, we could understand how variations in web structure influence the survival and reproductive success of their makers.



396 CHAPTER TWENTY-ONE

Darwin proposed a mechanism to explain adaptation

Charles Darwin was a keen naturalist who observed many examples of structures and behaviors that seemed to be designed to assist the survival and reproductive success of their bearers. He was given an unprecedented opportunity to study the adaptations of organisms in various parts of the world when in 1831, his Cambridge University botany professor, John Henslow, recommended him as a naturalist to Captain Robert Fitzroy, who was preparing to sail around the world on the survey ship H.M.S. Beagle (Figure 21.1). Whenever possible during the voyage, Darwin (who was often seasick) went ashore to observe and collect specimens of plants and animals.

Darwin spent most of his time ashore in South America, where the species he saw differed strikingly from those of Europe. He also noted that the species of the temperate regions of South America (Argentina and Chile) were more similar to those of tropical South America (Brazil) than they were to European species. When he explored the Galapagos

fc

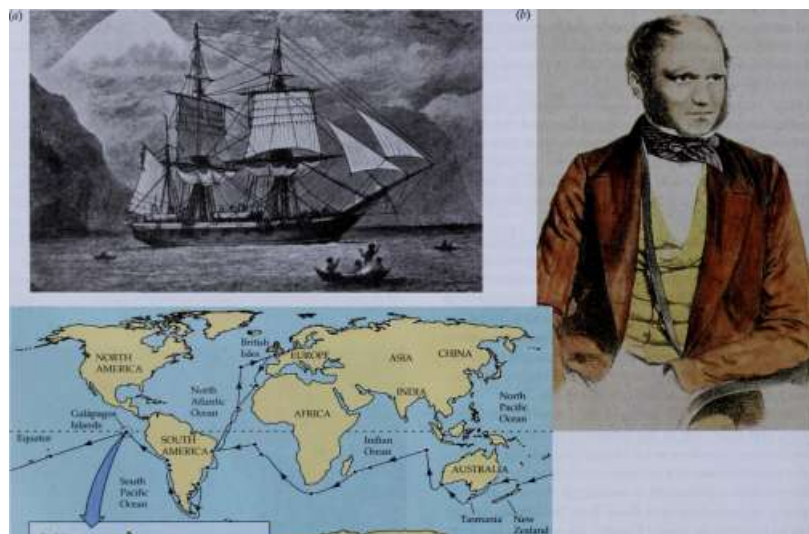
archipelago, west of Ecuador, he noted that most of its animal species were found nowhere else, but were similar to those of the mainland of South America, 1,000 kilometers to the east. Darwin also observed that the animals of the archipelago differed from island to island. He postulated that some animals had dispersed from mainland South America and then evolved differently on different islands.

When he returned to England in 1836, Darwin continued to ponder his observations. Within a decade he had developed the main features of his theory of evolution, which had two major components:

► Species are not immutable, but change, or adapt, over time. (In other words, Darwin asserted that evolution is a historical fact.)

* ► The agent that produces the changes is natural selection.

Darwin wrote a long essay on natural selection and the origin of species in 1844, but, despite urging from his wife and colleagues, he was reluctant to publish it, preferring to assemble more evidence first.



Galapagos Islands



Femandina

O Santiago

\ ^ Bartolome

v- ^ i Santa

Isabela /

ry San Cristobal

Tasmania New

Zealand

27.7 Darwin and the Voyage of the Beagle

(a) The mission of H.M.S. Beagle was to chart the oceans and collect oceanographic and biological information from around the world. The map indicates the ship's path, with emphasis on the Galapagos Islands, where his observations of the organisms he found were one source of Darwin's ideas on natural selection, (b) Charles Darwin at age 24, shortly after returning to England from the voyage of the Beagle.



27.2 Many Types of Pigeons Have Been Produced by Artificial Selection

Charles Darwin raised pigeons as a hobby, and he saw similar forces at work in artificial and natural selection. These are just some of over 300 varieties of pigeons that have been artificially selected by breeders to display different forms of traits such as color, size, and feather distribution.

Darwin's hand was forced in 1858 when he received a letter from Alfred Russel Wallace, who was studying plants and animals in the Malay Archipelago. Wallace asked Darwin to evaluate an enclosed manuscript, in which Wallace proposed a theory of natural selection almost identical to Darwin's. At first, Darwin was dismayed, believing that he had been preempted by Wallace. But extracts from Darwin's 1844 essay, together with Wallace's manuscript, were presented to the Linnaean Society of London on July 1, 1858, thereby giving credit for the idea to both men. Darwin then worked quickly to finish *The Origin of Species*, which was published the next year. Although both men conceived of natural selection independently, Darwin developed his ideas first, and his book provided a much more thorough justification of the concept—which is why natural selection is more closely associated with his name.

The facts that Darwin used to develop his theory of evolution by natural selection were familiar to most contemporary biologists. His insight was to perceive the significance of relationships among them. Darwin understood that populations of all species have the potential for exponential increases in numbers. To illustrate this point, he used the following example:

Suppose ... there are eight pairs of birds, and that only four pairs of them annually ... rear only four young, and that these go on rearing their young at the same rate, then at the end of seven years (a short life, excluding violent deaths for any bird) there will be 2,048 birds instead of the original sixteen.

Yet such rates of increase are rarely seen in nature. Therefore, Darwin knew that death rates in nature must be high. Without high death rates, even the most slowly reproducing species would quickly reach enormous population sizes.

Darwin also observed that, although offspring tend to resemble their parents, the offspring of most organisms are

not identical to one another or to their parents. He suggested that slight variations among individuals significantly affect the chance that a given individual will survive and the number of offspring it will produce. He called this differential reproduction the success of individuals in natural selection. Natural selection results from both differential survival and differential reproduction of individuals.

Darwin may have used the words "natural selection" because he was familiar with the artificial selection practices of animal and plant breeders. Many of Darwin's observations on the nature of variation came from domesticated plants and animals. Darwin was a pigeon breeder, and he knew firsthand the astonishing diversity in color, size, form, and behavior that could be achieved by humans selecting which pigeons to mate (Figure 21.2). He recognized close parallels between selection by breeders and selection in nature.

Darwin argued his case for natural selection in *The Origin of Species*:

How can it be doubted, from the struggle each individual has to obtain subsistence, that any minute variation in structure, habits or instincts, adapting that individual better to the new conditions, would tell upon its vigour and health? In the struggle it would have a better chance of surviving; and those of its offspring which inherited the variation, be it \

ever so slight, would have a better chance. /

That statement, written more than a hundred years ago, still stands as a good expression of the idea of evolution by natural selection.

Since Darwin wrote these words, biologists have developed a much deeper understanding of the genetic basis of evolutionary change and have assembled a rich array of examples of natural selection in action.

What have we learned about evolution since Darwin?

When Darwin proposed his theory of natural selection, he had no examples of selection operating in nature. He based his arguments on the results of selection on domesticated species. Since Darwin's time, many studies of the action of natural selection have been conducted; we will discuss some of them in this chapter.

398 CHAPTER TWENTY-ONE

We now know that biological evolution is a change over time in the genetic composition of a population. Darwin understood the importance of heredity for his theory, but he knew nothing of the mode of inheritance. He devoted considerable time to an attempt to develop a theory of heredity, but he failed to discover the laws of heredity, and he failed to understand the significance of Gregor Mendel's paper (see Chapter 10), which he apparently read.

Fortunately; the rediscovery of Mendel's publications in the 1900s paved the way for the development of population genetics, the field that provides a major underpinning for Darwin's theory. Population geneticists apply Mendel's laws to entire populations of organisms. They also study variation within and among species in order to understand the processes that result in evolutionary changes in species through time.

Genetic Variation within Populations

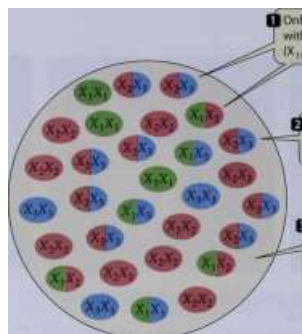
For a population to persist, its members must possess variation, which is the raw material on which agents of evolution act. In everyday life, we do not directly observe the genetic composition of organisms or populations. What we observe are phenotypes, the physical expressions of organisms' genes. The agents of evolution actually act on phenotypes, but for the moment we will concentrate on genetic variation within populations. We do so because genes are what is passed on to offspring via reproductive cells—eggs and sperm.

A heritable trait is a characteristic of an organism that is at least partly influenced by the organism's genes. The genetic constitution that governs a trait is called a genotype. A population evolves when individuals with different genotypes survive or reproduce at different rates.

Recall that different forms of a gene, called alleles, may exist at a particular locus. A single individual has only some of the alleles found in the population to which it belongs (Figure 21.3). The sum of all the alleles found in a population constitutes the gene pool. The gene pool contains the variation that produces the differing phenotypes on which agents of evolution act.

Fitness is the relative reproductive contribution of genotypes

The reproductive contribution of a genotype or phenotype to subsequent generations relative to the contribution of other genotypes or phenotypes in the same population is called fitness. The word "relative" is critical: The absolute number of offspring produced by an individual does not influence allele frequencies in the gene pool. Changes in absolute numbers of offspring are responsible for increases and decreases in the size of a population, but the relative success among genotypes within a population is what leads to changes in allele frequencies—that is, to evolution. When we discuss evolution, we talk about survival and reproductive success, because these rates determine how many genes different individuals contribute to subsequent generations.



Only locus X is shown, with three alleles (X_1 , X_2 and X_3).

No individual can have more than two of the alleles.

The gene pool is

the sum of all the alleles found in a population.

27.3 A Gene Pool

The allele proportions in this gene pool are 0.20 for X_1 , 0.50 for X_2 , and 0.30 for X_3 .

To contribute genes to subsequent generations, individuals must survive to reproductive age and produce offspring. The relative contribution of individuals of a particular genotype is determined by the probability that those individuals will survive times the average number of offspring they produce over their lifetimes. In other words, the fitness of a genotype is determined by the average rates of survival and reproduction of individuals with that genotype. |L I

Most populations are genetically variable

Some level of genetic variation characterizes nearly all natural populations. Such variation has been demonstrated repeatedly for thousands of years by people attempting to develop desirable traits in plants and animals. For example, selection for different traits in a European wild mustard produced many important crop plants (Figure 21.4). Plant and animal breeders can achieve such results only if the population has genetic variation for the traits of interest. Their success indicates that genetic variation is common, but it does not tell us how much variation there is.

Laboratory experiments also demonstrate that considerable genetic variation is present in most populations. In one such experiment, investigators chose as parents for subsequent generations of fruit flies (*Drosophila*) individuals with either high numbers or low numbers of bristles on their bodies. After 35 generations, flies in both lineages had bristle numbers that fell well outside the range found in the original population (Figure 21.5). These results show that there must have been considerable variation in the original fruit fly population for selection to act on.

To understand evolution, we need to know more precisely how much genetic variation populations contain, the sources of that genetic variation, and how genetic variation is maintained and expressed in populations in space and over time.

European agriculturalists chose as parents for subsequent generations individual wild mustard plants that varied from the population's average by producing unusually large leaves, stems, buds, or flowers.



THE MECHANISMS OF EVOLUTION 399

Brassica oleracea (a common wild mustard)



i^b



Cabbage

Brussels sprouts

Kohlrabi

Kale

Broccoli

Cauliflower

27.4 Many Vegetables from One Species

All of these crop plants have been derived from a single wild mustard species. They illustrate the vast amount of variation that can be present in a gene pool.

How do we measure genetic variation?

A locally interbreeding group within a geographic population is called a Mendelian population. Mendelian populations are often the subjects of evolutionary studies. To measure precisely the gene pool of a Mendelian population, we would need to count every allele at every locus in every organism in it. By measuring all the individuals, we could determine the relative proportions, or frequencies, of all alleles in the population.

Biologists can reliably estimate allele frequencies for a given locus by measuring numbers of alleles in a sample of individuals from a population. Measures of allele frequency range from 0 to 1; the sum of all allele frequencies at a locus is equal to 1. The frequencies of the different alleles at each locus and the frequencies of different genotypes in a Mendelian population describe its genetic structure.

An allele's frequency is calculated using the following formula:

$P =$

$\frac{\text{number of copies of the allele in the population}}{\text{sum of alleles in the population}}$

If only two alleles (for example, A and a) for a given locus are found among the members of a diploid population, they may combine to form three different genotypes: AA, Aa, and aa.

Low-selected population does not overlap original population after 35 generations.

Low-selected population



Original population



High-selected population does not overlap original population.



Abdominal bristles

High-selected population

J I L

10 20 30 40 50 60 70

Number of bristles

80

90

100

27.5 Artificial Selection Reveals Genetic Variation

In laboratory experiments with *Drosophila*, changes in bristle number evolved rapidly when selected for artificially.

400 CHAPTER TWENTY-ONE

Aa, and aa. Using the formula above, we can calculate the relative frequencies of alleles A and a in a population of N individuals as follows:

- Let N_{AA} be the number of individuals that are homozygous for the A allele (AA)
- Let N_{Aa} be the number that are heterozygous (Aa)
- Let N_{aa} be the number that are homozygous for the a allele (aa)

Note that $N_{AA} + N_{Aa} + N_{aa} = N$, the total number of individuals in the population, and that the total number of alleles present in the population is $2N$ because each individual is diploid. Each AA individual has two A alleles, and each Aa individual has one A allele. Therefore, the total number of A alleles in the population is $2N_{AA} + N_{Aa}$, and the total number of a alleles in the population is $2N_{aa} + N_{Aa}$.

If p represents the frequency of A, and q represents the frequency of a, then

$$2N_{AA} + N_{Aa} = 2Np$$

and

$$2N_{aa} + N_{Aa} = 2Nq$$

IN

$$2N_{AA} + N_{Aa} = 2Np$$

$$2N_{aa} + N_{Aa} = 2Nq$$

To show how this works, Figure 21.6 calculates the allele frequencies in two different populations, each containing 200 diploid individuals. Population 1 has mostly homozygotes (90 AA, 40 Aa, and 70 aa); population 2 has mostly heterozygotes (45 AA, 130 Aa, and 25 aa).

The calculations in Figure 21.6 demonstrate two important points. First, notice that for each population, $p + q = 1$. If there is only one allele in a population, its frequency is 1. If an allele is missing from a population, its frequency is 0, and the locus in that population is represented by one or more other alleles. Because $p + q = 1$, $q = 1 - p$, which means that when there are two alleles at a locus in a population, we can calculate the frequency of one allele and then easily obtain the second frequency by subtraction.

The second thing to notice in these calculations is that population 1 (consisting mostly of homozygotes) and pop-

ulation 2 (consisting mostly of heterozygotes) have the same allele frequencies for A and a. Therefore, they have the same gene pool for this locus. However, because the alleles in the gene pool are distributed differently, the genotype frequencies of the two populations differ.

Although we began our calculations with numbers of genotypes, for many purposes, genotype frequencies are best thought of as allele frequencies. Genotype frequencies are calculated as the number of individuals that have the genotype divided by the total number of individuals in the population. In population 1 of our example, the genotype frequencies are 0.45 AA, 0.20 Aa, and 0.35 aa.

ro



The Hardy-Weinberg Equilibrium

A population that is not changing genetically—that has the same allele and genotype frequencies from generation to generation—is said to be at Hardy-Weinberg equilibrium. The conditions that result in such an equilibrium population were discovered independently by the British mathematician Godfrey H. Hardy and the German physician Wilhelm Weinberg in 1908. Hardy wrote his equations in response to a question posed to him by the Mendelian geneticist Reginald C. Punnett (the inventor of the Punnett square) at the Cambridge University faculty club. Punnett was puzzled by the fact that although the allele for short fingers in humans was dominant and the allele for normal-length fingers was recessive, most people in Britain had normal-length fingers.

Hardy's equations explain why dominant alleles do not replace recessive alleles in populations. They also explain other features of the genetic structure of populations. The equations apply to sexually reproducing organisms. The particular example we will illustrate assumes that the organism in question is diploid, its generations are nonoverlapping, the gene

under consideration has two alleles, and allele frequencies are identical in males and females. The equilibrium also applies if there are more than two alleles and generations overlap, but in those cases the mathematics are more complicated.

In any population:

Frequency $\frac{2N_{AA} + N_{Aa}}{2N}$

of allele A " $P = \frac{2N_{AA} + N_{Aa}}{2N}$

Frequency $\frac{2N_{aa} + N_{Aa}}{2N}$

of allele a

where N is the total number of individuals in the population.

$2N$

For population 1 (mostly homozygotes): $N/M = 90 / N_A = 40$, and $N_W = 70$

so

$180 + 40 = 220$

$P = \frac{180}{220} = 0.818$

400

$140 + 40 = 180$

$= 0.45$

For population 2 (mostly heterozygotes):

$N_{AA} = 45, N_{Aa} = 130$, and $N_{aa} = 25$

so

$90 + 130 = 220$

$p = \frac{90}{220} = 0.409$

$q = 1 - p = 0.591$

400

$50 + 130 = 180$

0.45

27.6 Calculating Allele Frequencies

The gene pool and allele frequencies are the same for both populations, but the alleles are distributed differently between heterozygous and homozygous genotypes. In all cases, $p + q$ must equal 1.

Generation I



Genotypes

Frequency of

genotypes in population

Frequency of alleles in population

Generation II

$AA = 0.43$

$0.45 + 0.10$

V_J

Y

$$V = 0.55$$

$$An\ 0.20$$

$$aa$$

$$0.35$$

$$0.10 + 0.35$$

$$V \dots J$$

$$Y$$

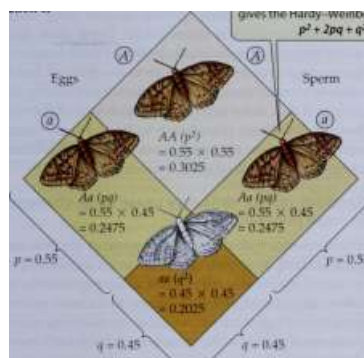
$$q = 0.45$$

$$\textcircled{R}$$

Gametes

$$\textcircled{R}$$

Adding the four genotype frequencies the Hardy-Weinberg equation: $= 1$



21.7 Calculating Hardy-Weinberg Genotype Frequencies

The areas within the squares are proportional to the expected frequencies of possible matings if mating is random with respect to genotype. Because there are two ways of producing a heterozygote, the probability of this event occurring is the sum of the two Aa squares.

To see why these results are true, consider population 1, used as an example in the previous section, in which the frequency of the A allele (p) is 0.55. Because we assume that individuals select mates at random, without regard to their genotype, gametes carrying A or a combine at random—that is, as predicted by the frequencies p and q. The probability that a particular sperm or egg in this example will bear an A allele rather than an a allele is 0.55. In other words, 55 out of 100 random selections of a sperm or an egg will bear an A allele. Because $q = 1 - p$, the probability of an a allele is $1 - 0.55 = 0.45$. To obtain the probability of two A-bearing gametes coming together at fertilization, we multiply the two independent probabilities of drawing them (see the discussion of probability in Chapter 10):

The essential assumptions that must be met for Hardy-Weinberg equilibrium are:



- Mating is random.
- Population size is very large.
- There is no migration between populations.
- Mutation can be ignored.
- Natural selection does not affect the alleles under consideration.

If these conditions hold, two results follow. First, the frequencies of alleles at a locus will remain constant from generation to generation. And second, after one generation of random mating, the genotype frequencies will remain in the proportions

Genotype: AA \ Aa

Frequency: p^2

Stated another way, this is the equation for Hardy-Weinberg equilibrium:

$$r + M + <r = i$$

$$p^2 = p^2 = (0.55)^2 = 0.3025$$

Therefore, 0.3025, or 30.25 percent, of the offspring in the next generation will have the AA genotype. Similarly, the probability of bringing together two rt-bearing gametes is

$$q^2 = q^2 = (0.45)^2 = 0.2025$$

so 20.25 percent of the next generation will have the aa genotype (Figure 21.7).

Figure 21.7 also shows that there are two ways of producing a heterozygote: an A sperm may combine with an a egg, the probability of which is $p \times q$; or an a sperm may combine with an A egg, the probability of which is $q \times p$. Consequently, the overall probability of obtaining a heterozygote is $2pq$.

It is now easy to show that allele frequencies p and q remain constant for each generation. Notice that the total of $p^2 + 2pq + q^2$ represents the total of the A alleles. The fraction that this frequency constitutes of all alleles is

$$\frac{p^2 + 2pq}{p^2 + 2pq + q^2}$$

$$= \frac{p^2 + 2pq + q^2}{p^2 + 2pq + q^2}$$

$$= \frac{p(p + q)}{p + q}$$

$$= p$$

$$p + (1 - p)$$

$p + q$ Similarly, the frequency of a in the next generation will be

$$= q$$

$$q + pq$$

$$p^2 + 2pq + q^2$$

$$(p + q)(p + q) = p + q$$



Thus the original allele frequencies are unchanged, and the population is at Hardy-Weinberg equilibrium.

If some agent, such as nonrandom mating, were to alter the allele frequencies, the genotype frequencies would automatically settle into a predictable new set in the next generation. For instance, if only AA and Aa individuals breed, p^2 would change, but there would still be aa individuals in the population.

402 CHAPTER TWENTY-ONE

Why is the Hardy-Weinberg equilibrium important?

The most important message of the Hardy-Weinberg equilibrium is that allele frequencies remain the same from generation to generation unless some agent acts to change them. Hence, simply because normal-length fingers in humans are recessive, we don't expect the frequency of normal-length fingers in the population to change unless a specific evolutionary force is acting on the underlying genes. The equilibrium also shows us what distribution of genotypes is expected for a population at genetic equilibrium at any value of p and q .

You may already have realized that populations in nature rarely meet the stringent conditions necessary to maintain them in Hardy-Weinberg equilibrium. Why, then, is it considered so important for the study of evolution? The answer is that without it, we cannot tell whether evolutionary agents are operating. More importantly, the pattern of deviations from the equilibrium tells us which assumptions are violated; thus we can identify the agents of evolutionary change on which we should concentrate our attention.

Microevolution: Changes in the Genetic Structure of Populations

Evolutionary agents are forces that change the allele and genotype frequencies in a population. In other words, they cause deviations from the Hardy-Weinberg equilibrium. Because such changes in the gene pool of a population constitute small-scale evolutionary changes, they are referred to as microevolution. The known evolutionary agents are mutation, gene flow, random genetic drift, nonrandom mating, and natural selection. Although only natural selection results in adaptation, to understand microevolutionary processes we need to discuss all five evolutionary agents before considering in detail how natural selection is studied.

[Mutation are changes in genetic material

The origin of genetic variation is germ-line mutations (see Chapter 12). These mutations appear to be random with respect

to the adaptive needs of organisms. Most mutations are harmful or neutral to (do not affect) their bearers. If the environment changes, however, previously harmful or neutral alleles may become advantageous.

Mutation rates are very low for most loci that have been studied. Rates as high as one mutation per locus in a thousand zygotes per generation are rare; one in a million is more typical. Nonetheless, these rates are sufficient to create considerable genetic variation because each of a large number of genes may mutate, and populations often contain a large number of individuals. For example, if the probability of mutation is 10^{-9} per nucleotide base pair per generation, then in each human gamete, the DNA of which contains 3×10^9 base pairs, there would be an average of one new mutation in each generation. Each newly fertilized egg would carry, on average, two new mutations. Therefore, the current human population of about 6 billion people would be expected to carry about 12 billion new mutations.

Mutations that were not present one generation earlier. In addition, mutations can restore to populations alleles that other evolutionary agents remove. Thus mutations both create and help maintain variation within populations.

One condition for Hardy-Weinberg equilibrium is that there be no mutation. Although this condition is never strictly met, the rate at which mutations arise at single loci is usually so low that mutations result in only very small deviations from Hardy-Weinberg expectations. If large deviations are found, it is appropriate to dismiss mutation as the cause and to look for evidence of other evolutionary agents.

Migration of individuals followed by breeding

produces gene flow

Because few populations are completely isolated from other populations of the same species, usually some migration between populations takes place. Gene flow happens when migrating individuals breed in their new location. Immigrants may add new alleles to the gene pool of a population, or may change the frequencies of alleles already present if they come from a population with different allele frequencies. For a population to be in Hardy-Weinberg equilibrium, there must be no immigration from other populations with different allele frequencies.

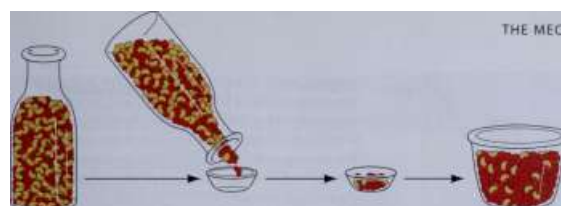
Random genetic drift may cause large changes in small populations

Chance events that alter allele frequencies result in random genetic drift. This process occurs at all loci in all populations, but has its greatest effect in small populations. If only a few individuals contribute genes to the next generation, the alleles they carry are not likely to be in the same proportions as alleles in the gene pool from which they were drawn.

In very small populations, random genetic drift may be strong enough to influence the direction of change of allele frequencies even when other evolutionary agents are pushing the frequencies in a different direction. Harmful alleles, for example, may increase because of random genetic drift, and rare advantageous alleles may be lost. As we will see later, even in large populations, random genetic drift can influence the frequencies of traits that do not influence the survival and reproductive rates of their bearers.

Even organisms that normally have large populations may pass through occasional periods when only a small number of individuals survive. During these population bottlenecks, genetic variation can be reduced by genetic drift. How this works is illustrated in Figure 21.8, in which allele frequencies are represented by red and yellow beans. In the small sample taken from the bean population, most of the beans that "survive" to germinate the next generation are, just by chance, red, so the new population has a much higher frequency of red beans than the previous generation had.

Suppose we have performed a cross of $Aa \times Aa$ individuals of a species of *Drosophila* to produce an offspring pop-

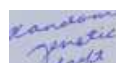


The original population has approximately equal frequencies of red and yellow alleles.

o A chance environmental event greatly reduces the population size.

Q The surviving individuals have different allele frequencies from the original population...

o ■ • .which generates a new population with more red than yellow alleles.



y^{tf*}

21.8 The Bottleneck Effect

Population bottlenecks occur when only a few individuals survive a random event, resulting in a shift in allele frequencies within the population.

ulation in which $p = q = 0.5$ and in which the genotype frequencies are 0.25 AA, 0.50 Aa, and 0.25 aa. If we randomly select four individuals from among the offspring to form the next generation, the allele frequencies in this small sample may differ markedly from $p = q = 0.5$. If, for example, we happen by chance to draw two AA homozygotes and two heterozygotes (Aa), the genotype frequencies in this "surviving population" are $p = 0.75$ and $q = 0.25$. If we replicate this sampling experiment 1,000 times, one of the two alleles will be missing entirely from about 8 of the 1,000 "surviving populations."

Populations in nature pass through bottlenecks for many different reasons. Predators may reduce populations of their prey to very small sizes. During the 1890s, hunting reduced the number of northern elephant seals to about 20 animals in a single population on the coast of Mexico. The actual breeding population may have been even smaller because in this species, only a few males mate with all the females and father all the offspring in any generation (Figure 21.9).

Using electrophoresis (see Chapter 17), investigators examined 24 proteins from tissues collected from the current California population of northern elephant seals. They found no evidence of variation in any of the 24 proteins. By contrast, the southern elephant seal, whose numbers were not severely reduced by hunting, has much more genetic variation. Currently, northern elephant seal populations are expanding rapidly, so their reduced genetic variation is not preventing high survival and reproductive rates. However, biologists worry that it may make them vulnerable to a disease outbreak or other sudden environmental change.

When a few pioneering individuals colonize a new re-

21.9 A Species with Low Genetic Variation

Because a few males sire most of the offspring in this northern elephant seal breeding colony, the size of the breeding population is smaller than the population as a whole. This pattern of non-random mating, together with a bottleneck that occurred when the seals were overhunted in the late nineteenth century, resulted in a population with very little genetic variation.



gion, the resulting population will not have all the alleles found among members of its source population. The resulting pattern of genetic variation, called a founder effect, is equivalent to that in a large population reduced by a bottleneck. Because individuals of many plant species can reproduce sexually by self-fertilization, a new plant population may be started by a single seed—an extreme example of a founder effect.



Scientists were given an opportunity to study the genetic composition of a founding population when *Drosophila sub-obscura*, a well-studied European species of fruit fly, was discovered near Puerto Montt, Chile, in 1978 and at Port Townsend, Washington, in 1982. In both South and North America, populations of the flies grew rapidly and expanded their ranges. Today in North America, *D. subobscura* ranges from British Columbia, Canada, to central California. In Chile it has spread across 23° of latitude, nearly as wide a range as the species has in Europe (Figure 21.10).

The *D. subobscura* founders probably reached Chile and the United States from Europe aboard the same ship, because both populations are genetically very similar. For example, the North and South American populations have only 20 chromosomal inversions, 19 of which are the same on the two continents, whereas 80 inversions are known from European populations. New World populations also have lower enzyme diversity than Old World populations. Only alleles that have a frequency higher than 0.1 in Euro-

Mirounga angustirostris



European populations of *D. subobscura* have 80 inversions.

| These populations of *D. subobscura* are very similar, but have only 20 inversions.



27.70 A Founder Effect

Populations of the fruit fly *Drosophila subobscura* in North and South America contain less genetic variation than the European populations from which they came, as shown by their numbers of chromosome inversions. Within two decades of arriving in the New World, the flies have increased dramatically and spread widely in spite of their reduced genetic variation.

European populations are present in the Americas. Thus, as expected from a small founding population, only a small part of the total genetic variation found in Europe reached the Americas. Geneticists estimate that at least ten, but no more than a hundred, flies initially arrived in the New World.

Nonrandom mating changes the frequency of homozygotes

Another Hardy-Weinberg assumption is that mating is random. In many cases, however, individuals with certain genotypes mate more often with individuals of either the same or different genotypes than would be expected on a



random basis. When such assortative mating takes place, the proportions of homozygotes and heterozygotes in the next generation differ from Hardy-Weinberg expectations. If individuals mate preferentially with other individuals of the same genotype, homozygous genotypes are overrepresented and heterozygous genotypes underrepresented in the next generation.

Alternatively, individuals may mate primarily or exclusively with individuals of a different genotype. An example is provided by plant species such as *Primula* (primroses) that have flowers of two types. One type, known as pin, has a tall style (female reproductive organ) and short stamens (male reproductive organs). The other type, known as thrum, has a short style and tall stamens (Figure 21.11). Pollen grains from pin and thrum flowers are deposited on different parts of the bodies of insects that visit the flowers. When the insects visit other flowers, pollen grains from pin flowers are most likely to come into contact with stigmas of thrum flowers, and vice versa. In most species with this reciprocal arrangement, pollen from one flower type can fertilize only flowers of the other type.

Self-fertilization (selfing), another form of nonrandom mating, is common in many groups of organisms, especially plants. Selfing reduces the frequencies of heterozygous individuals below Hardy-Weinberg expectations. Under assortative mating and self-fertilization, genotype frequencies change, but allele frequencies remain the same.

\r?

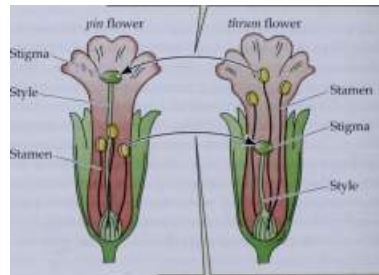


Natural selection produces variable results

As we have seen, individuals vary in heritable traits that determine the success of their reproductive efforts. Not all

f

Insect pollinators that work high on flowers pick up mostly thrum pollen and deposit it on pin stigmas.



Stamen

?

Insects working low in flowers pick up pin pollen and deposit it on thrum stigmas.

Primula sinensis

21.11 Floral Structure Fosters Assortative Mating

The structure of flowers in species such as the primroses (*Primula*) assures that fertilization usually occurs between individuals of different types.

THE MECHANISMS OF EVOLUTION 405

Stabilizing selection reduces variation but does not change the mean.

Directional selection changes the mean value of the trait (in this case toward larger size).

Disruptive selection favors both extremes and produces two peaks in the distribution of a trait.

a

73. o.

> >?

~5 2

o a.

I r*

% ■ %

3

Z



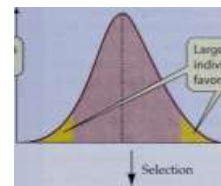
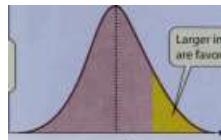
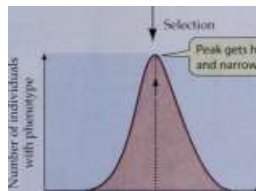
Medium-sized individuals are favored.

Phenotypes favored by natural selection are shown in yellow.

Larger individuals are favored.

Selection

Peak gets higher and narrower.



Large and small individuals are favored.

Selection

Two peaks form.



^



Body size

27.12 Natural Selection Operates on a Variable Trait

The curves plot the distributions of body size in a population before selection (top) and after selection (bottom). Natural selection may change the shape and position of the original curves.

Individuals survive and reproduce equally well in a particular environment. Therefore, some individuals contribute more offspring to the next generation than others. This process is known as natural selection, and it causes allele frequencies in the population to change.

Depending on which traits are favored in a population, natural selection can produce any one of several quite different results.

- Selection may preserve the characteristics of a population by favoring average individuals.
- Selection may change the characteristics of a population by favoring individuals that vary in one direction from the mean of the population.
- Selection may change the characteristics of a population by favoring individuals that vary in both directions from the mean of the population.

Until now, we have been considering traits influenced by alleles at only a single locus. However, most traits are influenced by alleles at more than one locus. The size of an organism, for example, is likely to be controlled by many different loci. If many loci influence size—and there is no selection—then the distribution of sizes in a population should approximate the bell-shaped curve shown in the top row of Figure 21.12.

stabilizing selection. If both the smallest and the largest individuals contribute relatively fewer offspring to the

next generation than those closer to the average size do, stabilizing selection is operating (Figure 21.12a). Stabilizing selection reduces variation, but does not change the mean. Natural selection frequently acts in this way, countering increases in variation brought about by mutation or migration. We know from the fossil record that most populations evolve slowly most of the time. Rates of evolution are typically very slow because natural selection is usually stabilizing.

Biologists measured the results of the action of natural selection on cliff swallows in Nebraska. These birds nest in dense colonies (Figure 21.13f). They eat flying insects, so can feed only when weather conditions permit flying insects to be active. In 1996, a severe cold spell, which began on May 24 and lasted for 6 days, killed thousands of birds and reduced the local population by about 53 percent. During the first 3 days after the cold spell, the investigators collected nearly 2,000 dead cliff swallows underneath their nesting colonies, and also captured about 1,000 birds that had survived the cold spell.

By carefully measuring the sizes and shapes of these birds, the investigators were able to show that larger birds survived better during the cold spell than smaller birds (Figure 21.13b). They also found that birds whose wings and tails were more symmetrical survived better than individuals with greater wing and tail asymmetry (Figure 21.13c). Larger swallows probably survived better because they had more favorable surface-to-volume ratios and were able to store more fat. Birds with symmetrical wings and tails were probably more maneuverable, and hence more efficient at capturing flying insects. So stabilizing selection maintains a high level of wing and tail symmetry, and (because cliff

406 CHAPTER TWENTY-ONE

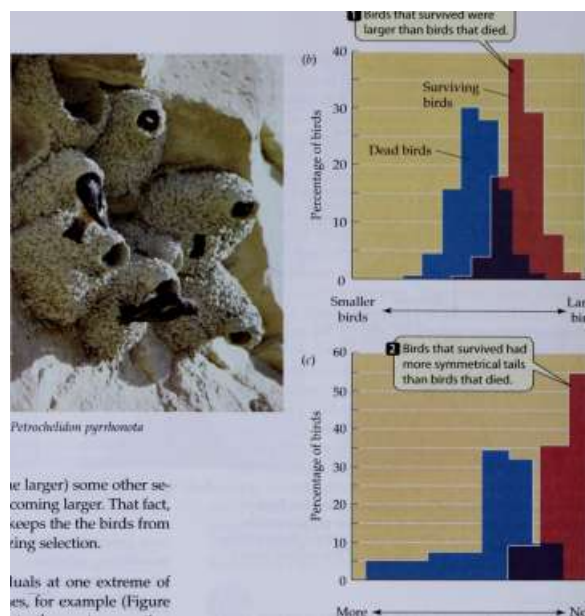
f

Birds that survived were larger than birds that died.

21.13 Size and Symmetry Are Selected in Cliff Swallows

(a) Cliff swallows build their mud nests in dense colonies. Larger (b) and more symmetrical (c) birds survived better during a period of cold weather.

00



Larger birds

^^■^

Petrochelidon pyrrhonota

Wi

W

swallows are not evolving to become larger) some other selective force keeps the birds from becoming larger. That fact, together with cold weather (which keeps the the birds from becoming smaller), results in stabilizing selection.

directional selection. If individuals at one extreme of the size distribution—the larger ones, for example (Figure 21.12b)—contribute more offspring to the next generation than other individuals do, then the mean size of individuals in the population will increase. In this case, directional selection is operating.

If directionaTselection operates over many generations, an evolutionary trend within the population results. Such directional evolutionary trends often continue for many generations, but they may be reversed if the environment changes and different phenotypes are favored, or they may be halted if an optimum is reached and the character then falls under stabilizing selection. The rapid evolution of rejection of parasite eggs by magpies, which we discussed at the beginning of the chapter, is an example of directional selection.

disruptive selection. Disruptive selection is selection that simultaneously favors individuals at both extremes of the distribution (Figure 21.12c). This type of selection apparently is rare. When disruptive selection operates, individuals at the extremes contribute more offspring than those in the center, producing two peaks in the distribution of a trait. The strikingly

bimodal (two-peaked) distribution of bill sizes in the black-bellied seedcracker, *Pyrenestes ostrinus*, a West African finch (Figure 21.14), illustrates how disruptive selection can adapt populations in nature. Seeds of two types of sedges (a marsh plant) are the most abundant food source for the finches during part of the year. Birds with large bills can readily crack the hard seeds of the sedge *Scirpus verrucosa*. Birds with small bills can crack *S. verrucosa*

&

► None

Amount of asymmetry

seeds only with difficulty, but they can feed more efficiently on the soft seeds of the other sedge, *S. goossensii*, than birds with larger bills.

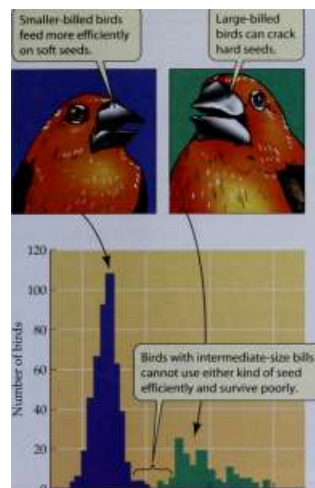
Young finches whose bills deviate markedly from the two predominant bill sizes do not survive as well as finches whose bills are close to one of the two sizes represented by the distribution peaks. Because there are few abundant food sources in the environment and because the seeds of the two sedges do not overlap in hardness, birds with intermediate-sized bills are inefficient in utilizing either one of the principal food sources. Disruptive selection therefore maintains a bill size distribution with two peaks.

Studying Microevolution

Biologists use several different methods to study microevolution, as illustrated by the examples we have just discussed. One method is to measure survival in the field under varying environmental conditions, as was done with cliff swallows. Another is to alter genotypes or phenotypes artificially and compare the performance of altered and normal individuals. A third is to use computer models to simulate natural selection. Here we discuss examples of the latter two methods.

Smaller-billed birds feed more efficiently on soft seeds.

Large-billed birds can crack hard seeds.



12 14 16 18

Width of lower bill (mm)

27.74 Natural Selection Alters Bill Sizes

The bimodal distribution of bill sizes in the black-bellied seed-cracker of West Africa is an example of disruptive selection, which favors individuals with larger and smaller bill sizes over individuals with intermediate-sized bills.

ALTERING GENOTYPES AND COMPARING PERFORMANCE. Modern

farmers attempt to control weeds by applying herbicides to crops. The success of this method is often poor because natural selection favors plants that produce chemicals that confer resistance to herbicides. But producing these chemicals is costly for the plant. Estimating the cost of defense against herbicides is difficult because individuals that differ in the kinds and concentrations of defensive chemicals they produce also differ in many other ways.

A powerful method of isolating the costs of producing and maintaining a specific resistance-conferring compound uses plasmids to transfer recombinant DNA into plants (see Chapter 17). The cost associated with resistance to the herbicide chlorosulfuron, conferred by a single allele, was measured in *Arabidopsis thaliana*. This allele, *Csrl-1*, results in the production of an enzyme that is insensitive to chlorosulfuron. Researchers transferred the *Csrl-1* allele to some individuals; other, genetically identical individuals received empty plasmids. Plants with the *Csrl-1* allele produced 34 percent fewer seeds than the nonresistant plants when grown in the presence of the herbicide (Figure 21.15). The reason for the high cost is not fully known, but evidence suggests that the allele results in an accumulation of branched-chain amino acids that interfere with metabolism.

computer modeling OF spider webs. Many species of spiders construct webs of sticky silk with which they capture flying insects. Because spider webs are relatively simple, two-dimensional structures, they are easy to model with computers. One such computer model, called NetSpinner, builds "webs" on a computer screen using behavioral rules that are actually used by web-building spiders. The model assumes that these behavioral rules are inherited, allowing them to be altered by "mutations." In each generation of the model, six spiders build webs, each using slightly different web-building rules. NetSpinner then shoots "flies" randomly at the "webs" and counts how many are captured. The quality of a web is calculated as the number of "flies" it captures, minus its cost, which is assessed by the length of silk used to make it. A fraction of the population of spiders—those that made the least efficient webs—dies every generation. The remaining spiders mate with one another at random to produce a new generation of spiders.

An example of the webs that emerged from a run of 40 generations of NetSpinner is shown in Figure 21.16. These webs are remarkably similar to those of real web-spinning spiders. Although such computer models do not measure

EXPERIMENT

Question: Is there a reproductive cost of producing and maintaining generic resistance to an herbicide?

METHOD Use a plasmid vector (see Figure 17.5) to insert an

allele that confers herbicide resistance. Control plants received the same vector without the resistance gene.

Treated

U—Resistance aUe.e ^J

Control

RESULTS

Untreated plants

without resistance

allele

Control plants

(receive empty

plasmid)

Plants receiving

allele that confers

resistance

There is no significant difference in seed production between untreated and control plants.

—



j_

10 15 20

Thousands of seeds

25

Resistant plants produced 34% fewer seeds.

Conclusion: The cost of producing and maintaining a single resistance-conferring compound is high.

21.15 Producing and Maintaining a Chemical Is Costly

Possession of a gene that confers resistance to an herbicide greatly reduced seed production in *Arabidopsis thaliana*.

Recombination in sexually reproducing organisms amplifies existing genetic variation. In asexually reproducing organisms, the cells resulting from a mitotic division normally contain identical genotypes. Each new individual is genetically identical to its parent unless there has been a mutation. When organisms exchange genetic material during sexual reproduction,

however, the offspring differ from their parents because chromosomes assort randomly during meiosis, crossing over occurs, and fertilization brings together material from two different cells (see Chapter 9).

Sexual reproduction generates an endless variety of genotypic combinations that increases the evolutionary potential of populations. Because it increases the variation among the offspring produced by an individual, sexual reproduction may improve the chance that at least some of the offspring will be successful in the varying and often unpredictable environments they will encounter. Sexual reproduction does not influence the frequencies of alleles; rather, it generates new combinations of alleles on which natural selection can act. It expands variation in a trait influenced by alleles at many loci by creating new genotypes. That is why selection for bristle number in *Drosophila* (see Figure 21.5) resulted in flies with more bristles than any flies in the initial population had.

Neutral genetic mutations accumulate within species

As we saw in Chapter 12, some mutations do not affect the functioning of the proteins the mutated genes encode. An allele that does not affect the fitness of an organism is called a neutral allele, but a mutation touched by natural selection, may be lost or their frequencies may increase with time. Therefore, neutral alleles tend to accumulate in a population over time, providing it with considerable genetic variation.

Much of the variation in those traits we can observe with our unaided senses is not neutral. However, much variation at the molecular level apparently is neutral. Modern molecular techniques enable us to measure variation in neutral traits and provide a means by which we can distinguish adaptive from neutral variation. Chapter 24 will discuss how these techniques enable us to make such discriminations, and how variation in neutral traits can be used to estimate rates of evolution.

Much genetic variation is maintained in geographically distinct subpopulations

Much of the genetic variation in large populations is preserved as differences among subpopulations. Subpopulations often vary genetically because they are subjected to different selective pressures in different environments. Plant subpopulations, for example, may vary geographically.

The proportion of cyanide-producing individuals increases gradually (clinally) along a gradient from colder to milder winters.



These white lines connect points with equal January mean temperatures.



«C

White indicates proportion not producing cyanide

Red indicates proportion producing cyanide

THE MECHANISMS OF EVOLUTION 409

that of other genotypes (or phenotypes). This process is known as frequency-dependent selection.

A small fish that lives in Lake Tanganyika in east central Africa provides an example of frequency-dependent selection. The mouth of this scale-eating fish, *Perissodus microlepis*, opens either to the right or to the left as a result of an asymmetrical jaw joint (Figure 21.18). *Perissodus* approaches its prey (another fish) from behind and dashes in to bite off several scales from its flank. "Right-mouthed" individuals always attack from the victim's left; "left-mouthed" individuals always attack

from the victim's right. The distorted mouth enlarges the area of teeth in contact with the prey's flank, but only if the scale eater attacks from the appropriate side.

Prey fish are alert to approaching scale eaters, so attacks are more likely to be successful if the prey must watch both flanks. Guarding by the prey favors equal numbers of right-mouthed and left-mouthed scale eaters, because if one "form" were more common than the other, prey fish would pay more attention to potential attacks from the corresponding flank. Therefore, success of individuals of the more common morph would be less than that of the less common morph. Over an 11-year period, the polymorphism was found to be stable: the two forms of *Perissodus* remained at about equal frequencies.

21.17 Geographic Variation in Poisonous Clovers

The frequency of cyanide-producing individuals in each population of white clover (*Trifolium repens*) is represented by the proportion of the circle that is red.

cally in the chemicals they synthesize to defend themselves against herbivores. Some individuals of the clover *Trifolium repens* produce the poisonous chemical cyanide. Poisonous individuals are less appealing to herbivores—particularly mice and slugs—than are nonpoisonous individuals. However, clover plants with cyanide are more likely to be killed by frost, because freezing damages cell membranes and releases the toxic cyanide into the plant's own tissues, which

In populations of *Trifolium repens* the frequency of cyanide-producing individuals increases gradually from north to south and from east to west across Europe (Figure 21.17). Poisonous individuals make up a large proportion of clover populations only in areas where the winters are mild. Cyanide-producing individuals are rare where winters are cold, even though herbivores graze clovers heavily in those areas.

Frequency-dependent selection maintains genetic variation within populations

Natural selection often preserves variation as polymorphisms—genetic differences within a population. A polymorphism may be maintained when the fitness of a genotype (or phenotype) varies with its frequency relative to



■ ■

LA«J

Q "Right-mouthed" *Perissodus* attack prey from the left rear side.



§| "Left-mouthed"

Perissodus attack prey from the right rear side.

27.78 A Stable Polymorphism

Frequency-dependent selection maintains equal proportions of left-mouthed and right-mouthed individuals of the scale-eating fish *Perissodus microlepis*.

1 ea\ es of a white o.ik [Quercus nlnb)



Grown in sun

Grown in shade

27.79 Environmentally Induced Variation

Traits may vary among genetically identical individuals or parts of individuals if they are exposed to different environments.



27.20 One Genotype: Two Seasonal Color Forms

The dry-season (left) and wet-season (right) form of the butterfly *Bicyclus anynana* have the same genotype. The environmental conditions experienced by a larva determine the form of the butterfly into which it develops.

■ *

How Do Genotypes Determine Phenotypes?

Genotypes do not uniquely determine phenotypes. If one allele is dominant to another, a particular phenotype can be produced by more than one genotype (for example, AA and Aa individuals may be phenotypically identical).

Similarly, different phenotypes can be produced by a given genotype, depending on the environment encountered during development. For example, the cells of the leaves on a tree or shrub are normally genetically identical. Yet leaves on the same tree often differ in shape and size. Leaves close to the top of an oak tree, where they are exposed to more wind and sunlight, may be more deeply lobed than leaves lower down on the same tree (Figure 21.19). The same differences can be seen between the leaves of individuals growing in sunny and shady sites.

Thus, the phenotype of an organism is the outcome of a complex series of developmental processes that are influenced by both environmental factors and genes. This nearly universal phenomenon is called phenotypic plasticity. Although variations in leaf shapes are not passed on to offspring, the ability to produce varied leaf shapes in response to environmental conditions is inherited. Phenotypic plasticity of leaf shapes benefits a tree because deeply lobed leaves offer less resistance to wind, absorb less sunlight, lose heat more rapidly by convection, and allow more sunlight to pass to lower leaves.

Because phenotypic plasticity is often adaptive, it may evolve under the influence of natural selection. A particularly thorough demonstration of the adaptive nature of phenotypic plasticity is provided by studies of the tropical African butterfly *Bicyclus anynana*. *B. anynana*, which lives in areas with distinct wet and dry seasons, has two distinct forms (Figure 21.20). The dry-season form, which rests on dried grasses and leaf litter and flies infrequently, has only one small wing spot. The wet-season form, which flies actively in lush, green vegetation, has many conspicuous spots on its wings.

The form of an adult butterfly is determined by the environmental conditions it encounters as a larva. Investigators

<&

compared the survival rates of the two forms in both seasons. The dry-season form, which closely matches the brown vegetation on which it rests, survives better during the dry season than does the wet-season form. On the other hand, the more active, conspicuous wet-season form does better during the wet season because its conspicuous spots, which resemble large eyes, deter some predators.

Constraints on Evolution

Thus far we have implicitly assumed that sufficient genetic variation always exists for the evolution of favored traits. A moment's reflection reveals that this assumption cannot be true. As we pointed out in the previous chapter, major evolutionary innovations are rare. Most changes are based on modifications of previously existing traits, even though those

traits may come to serve new functions. In addition, evolutionary theory does not allow a population to temporarily become less well adapted. All intermediate forms must work; that is, all modifications must benefit their bearers in every generation.

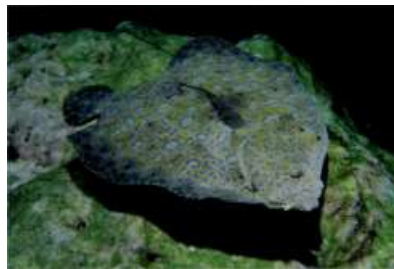
~K striking example that illustrates how natural selection operates by modifying existing states is provided by the evolution of fishes that spend most of their time resting on the sea bottom. One lineage, the bottom-dwelling skates and rays, is strikingly symmetrical (Figure 21.21a). These fishes are descended from sharks, which were already somewhat flattened and, therefore, able to lie on their bellies.

Plaice, sole, and flounders, on the other hand, are descendants of deep-bodied ancestors. Unlike sharks, these fishes cannot lie on their bellies; they must flop over on their sides. During development, the eyes of plaice and sole are grotesquely twisted around to bring both eyes to one side of the body (Figure 21.21b). No clever designer who was free of constraints would have designed plaice and sole as they are. But small shifts in the position of one eye probably helped ancestral flatfishes see better, resulting in the form found today.

Although constraints on evolution clearly exist, it is difficult to determine whether the absence of certain traits that



(a) *Taeniura lymma*



(b) *Bothus lunatus*

21.21 Two Solutions to a Single Problem

(a) Stingrays, whose ancestors were dorsally flattened, lie on their bellies, (b) Flounders, whose ancestors were laterally flattened, lie on their sides.

would seem to be desirable is due to some constraint or to our having wrongly guessed that the trait would be adaptive. A plausible answer to this question is now available for a puzzling pattern among amphibians. Many salamanders are neotenic; that is, individuals become sexually mature while still in their aquatic larval form. Why, then, are there no frogs and toads that reproduce when they are tadpoles? A universal constraint preventing frogs and toads from evolving neoteny may be their need for relatively high levels of thyroid hormones for sex differentiation and reproduction. Neotenic salamanders result only when levels of thyroid hormones are very low—too low to allow frogs and toads to become sexually mature. In many other cases, however, no plausible answer is yet available.

Short-Term versus Long-Term Evolution

Microevolutionary changes within populations are an important focus of study for evolutionary biologists. These changes can be observed directly, they can be manipulated experimentally, and they show the actual processes by which evolution occurs. Studies of these short-term changes identify the genetic bases of evolutionary changes

THE MECHANISMS OF EVOLUTION 411

and demonstrate how natural selection acts. By themselves, however, they do not enable us to predict—or, more properly, "postdict" (because they have already happened)—the macroevolutionary changes we described in Chapter 20.

The reason for this is that patterns of macroevolutionary change can be strongly influenced by events that occur so infrequently or so slowly that they are unlikely to be observed during microevolutionary studies. In addition, the ways in which evolutionary agents act may change with time; even among the descendants of a single ancestral species, different lineages may evolve in different directions. Therefore, additional types of evidence, such as the occurrence of rare and unusual events and trends in the fossil record, must be gathered in order to understand the course of evolution over billions of years.

"Postdiction" problems in science are not unique to evolutionary studies. For example, volcanologists believe they understand the physical theory that explains why Mount St. Helens erupted in 1980, but they lack the detailed information

necessary for them to "explain" why the mountain erupted on the exact day it did. Similarly, even though seismologists know the physical principles that govern earthquakes, they cannot predict exactly when or where an earthquake will happen.

In subsequent chapters we will discuss the kinds of information that biologists assemble to study long-term evolutionary changes and infer the processes that led to them.

Chapter Summary

Charles Darwin and Adaptation

- ▶ Darwin developed his theory of evolution by natural selection by carefully observing nature, especially during his voyage around the world on the Beagle.
- ▶ Darwin based his theory on well-known facts and some key inferences.
- ▶ Darwin had no examples of the action of natural selection, so he based his arguments on artificial selection by plant and animal breeders.
- ▶ Modern genetics has elucidated the mechanisms of heredity, which were unknown to Darwin but which have provided the solid base that supports and substantiates his theory.

Genetic Variation within Populations

- ▶ A single individual has only some of the alleles found in the population of which it is a member. Review Figure 21.3
- ▶ Genetic variation characterizes nearly all natural populations. Review Figures 21.4, 21.5
- ▶ Allele frequencies measure the amount of genetic variation in a population. Genotype frequencies show how a population's genetic variation is distributed among its members.
- ▶ Biologists estimate allele frequencies by measuring a sample of individuals from a population. The sum of all allele frequencies at a locus is equal to 1. Review Figure 21.6
- ▶ Populations that have the same allele frequencies may nonetheless have different genotype frequencies. $p^2 + 2pq + q^2 = 1$

The Hardy-Weinberg Equilibrium

- ▶ A population that is not changing genetically is said to be at Hardy-Weinberg equilibrium.

p^2

412 CHAPTER TWENTY-ONE

- ▶ The assumptions that underlie the Hardy-Weinberg equilibrium are that the population is large, mating is random, there is no migration, mutation can be ignored, and natural selection is not acting on the population.
- ▶ In a population at Hardy-Weinberg equilibrium, allele frequencies remain the same from generation to generation. In addition, genotype frequencies remain in the proportions

$p^2 + 2pq + q^2 = 1$. Review Figure 21.7

- ▶ Biologists can determine whether an agent of evolution is acting on a population by comparing the genotype frequencies of that population with Hardy-Weinberg equilibrium frequencies.

Microevolution: Changes in the Genetic Structure of Populations

- ▶ Changes in allele frequencies and genotype frequencies within populations are caused by the actions of several evolutionary agents: mutation, gene flow, random genetic drift, assortative mating, and natural selection.
- ▶ The origin of genetic variation is mutation. Most mutations are harmful or neutral to their bearers, but some are advantageous, particularly if the environment changes.
- ▶ Migration of individuals from one population to another, followed by breeding in the new location, produces gene flow. Immigrants may add new alleles to a population or may change the frequencies of alleles already present.
- ▶ Random genetic drift alters allele frequencies in all populations, but it overrides natural selection only in small populations. Organisms that normally have large populations may pass through occasional periods (population bottlenecks) when only a small number of individuals survive. Review Figure 21.8
- ▶ New populations established by a few founding individuals also have gene frequencies that differ from those in the parent population. Review Figure 21.10
- ▶ If individuals mate more often with individuals that have the same or different genotypes than would be expected on a random basis—that is, when mating is not random—frequencies of homozygous and heterozygous genotypes differ from

Hardy-Weinberg expectations. Review Figure 21.11

- ▶ Self-fertilization, an extreme form of nonrandom mating, reduces the frequencies of heterozygous individuals below Hardy-Weinberg expectations without changing allele frequencies.
- ▶ Natural selection is the only agent of evolution that adapts populations to their environments. Natural selection may preserve allele frequencies or cause them to change with time.
- ▶ Stabilizing selection, directional selection, and disruptive selection change the distributions of phenotypes governed by more than one locus. Review Figures 21.12, 21.13, 21.14

Studying Microevolution

- ▶ Biologists study microevolution by measuring natural selection in the field, experimentally altering organisms, and building computer models. Review Figures 21.15, 21.16

Maintaining Genetic Variation

- ▶ Random genetic drift, stabilizing selection, and directional selection all tend to reduce genetic variation, but most populations are genetically highly variable.
- ▶ Sexual reproduction generates an endless variety of geno-typic combinations that increases the evolutionary potential of populations, but it does not influence the frequencies of alleles. Rather, it generates new combinations of genetic material on which natural selection can act.
- ▶ Much genetic variation within many species is maintained in distinct subpopulations. Review Figure 21.17
- ▶ Genetic variation within a population may be maintained by frequency-dependent selection. Review Figure 21.18

How Do Genotypes Determine Phenotypes?

- ▶ Genotypes do not uniquely determine phenotypes. A given phenotype can be produced by more than one genotype.
- ▶ The phenotype of an organism is the result of a complex series of developmental processes that are influenced by both environmental factors and genes. Review Figures 21.19, 21.20

Constraints on Evolution

- ▶ Natural selection acts by modifying what already exists. A population cannot get temporarily worse in order to achieve some long-term advantage.

Short-Term versus Long-Term Evolution

- ▶ Patterns of macroevolutionary change can be strongly influenced by events that occur so infrequently or so slowly that they are unlikely to be observed during microevolution-ary studies. Additional types of evidence must be gathered to understand why evolution in the long term took the particular course it did.

For Discussion

1. During the past 50 years, more than 200 species of insects that attack crop plants have become highly resistant to DDT and other pesticides. Using your recently acquired knowledge of evolutionary processes, explain the rapid and widespread evolution of resistance. Propose ways of using pesticides that would slow down the rate of evolution of resistance. Now that the use of DDT has been banned in the United States, what do you expect to happen to levels of resistance to DDT among insect populations? Justify your answer.
2. In what ways does artificial selection by humans differ from natural selection in nature? Was Darwin wise to base so much of his argument for natural selection on the results of artificial selection?
3. In nature, mating among individuals in a population is never truly random: Immigration and emigration are common, and natural selection is seldom totally absent. Why, then, does it make sense to use the Hardy-Weinberg model, which is based on assumptions known generally to be false? Can you think of other models in science that are based on false assumptions? How are such models used?
4. As far as we know, natural selection cannot adapt organisms to future events. Yet many organisms appear to respond to natural events before they happen. For example, many mammals go into hibernation while it is still quite warm. Similarly, many birds leave the temperate zone for their southern wintering grounds long before winter arrives. How can such "anticipatory" behaviors evolve?
5. Some people believe that species, like individual organisms, have life cycles. They believe that species are born by a process of speciation, grow and expand, and inevitably die out as a result of "species old age." Could any agents of evolution cause such a species life cycle? If not, how do you explain the high rates of extinction of species in nature?

??



During the 1940s, officials in Trinidad launched an intensive campaign to control malaria. Believing that malaria was being transmitted by *Anopheles albimanus*, a swamp-breeding mosquito that is the principal vector of malaria in Latin America, they spent a great deal of money spraying and draining marshes. The campaign failed, however, because the principal vector of malaria in Trinidad was *Anopheles bellator*, a mosquito species that breeds in water held within the leaves of bromeliad plants (relatives of pineapples) growing on tree branches.

Similarly, in Europe, people thought that malaria was transmitted only by mosquitoes of a single species: *Anopheles maculipennis*. European efforts to control malaria sometimes succeeded and sometimes failed, because *A. maculipennis* turned out to be not a single species, but a group of at least 15 species that can be distinguished only by examination of their chromosomes. Some of the species breed in fresh water, others in brackish water. Some enter houses, others do not. Furthermore, which mosquito species is the vector of malaria varies regionally. Control efforts are successful only when directed against the species that actually transmits malaria in that area.

Therefore, to control malaria, we need to know which species of mosquitoes are the vectors of the disease, as well as the details of their life cycles. But how did these many species of mosquitoes arise? What processes keep them cohesive and distinct?

All species, living and extinct, are believed to be descendants of a single ancestral species that lived more than 3 billion years ago. If speciation were a rare event, the biological world would be very different than it is today. Speciation is an essential ingredient of evolutionary diversification, and species are the fundamental units of the biological classification systems we will discuss in Chapter 23. But what are species? How did these millions of species form? How does one species become two? What factors stimulate such splitting? What conditions spur evolutionary radiations? These and related questions are the subject of this chapter.

Trinidad Rainforest

The mosquito that transmits malaria in Trinidad breeds in water held in the bases of leaves of bromeliad plants that grow on the trunks and branches of rainforest trees.

What Are Species?

The word species means, literally, "kinds." But what do we mean by "kinds"? Someone who is knowledgeable about a group of organisms, such as orchids or lizards, usually can distinguish the different species of that group found in a particular area simply by examining them superficially. The patterns of similarities and differences that unite groups of organisms and separate them from other groups are familiar to all of us. The standard field guides to birds, mammals, insects, and flowers are possible only because most species are cohesive units that change in appearance only gradually over large geographic distances. We can easily recognize red-winged blackbirds from New York and red-winged blackbirds from California as members of the same species (Figure 22.1).

But not all members of a species look that much alike. For example, males, females, and young individuals may not resemble one another. How do we decide whether similar but easily distinguished individuals should be assigned to different species or regarded as members of the same species? The concept that has guided these decisions for a long time is genetic integration. If individuals within a population mate with one another but not with individuals of





22.1 Redwings Are Redwings Everywhere

Both of these male birds are obviously red-winged blackbirds, even though (a) lives in the eastern United States and (b) lives in California. In parts of California, males have less yellow in their wings than males elsewhere in the broad range of the species.

(b) *Agelaius phoeniceus*

(a) *Agelaius phoeniceus*

other populations, they constitute a distinct group within which genes recombine; that is, they are independent evolutionary units. These independent evolutionary units are usually called species.

More than 200 years ago the Swedish biologist Carolus Linnaeus, who originated the system of naming organisms that we use today, described hundreds of species. Because he knew nothing about the mating patterns of the organisms he was naming, Linnaeus classified them on the basis of their appearances; in other words, he used a morphological concept of species. Many species that were classified by their appearances are actually independent evolutionary units. They look alike because they share many alleles that code for body structures. In many groups of organisms for which genetic data are unavailable, species are still recognized by their morphological traits.

A species definition that has been used by many biologists—the "biological" species definition—was proposed by Ernst Mayr in 1940. He stated, "Species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups." The words "actually or potentially" assert that, even if some members of a species are not in the same place and hence are unable to mate, they should not be placed in separate species if they would be likely to mate if they were together. The word "natural" is an important part of the definition because only in nature does the exchange of genes affect evolutionary processes; the interbreeding of two different species in captivity does not. Gene exchange is the main reason why species are cohesive units.

Deciding whether two populations constitute different species can be difficult because speciation is often a gradual process (Figure 22.2). If a barrier divides one population into two populations, the daughter populations may evolve independently long before they become reproductively incompatible—or they may become reproductively incompatible before they evolve any noticeable morphological differences.

[fill]



How Do New Species Arise?

Speciation is the process by which one species splits into two species, which thereafter evolve as distinct lineages. Not all evolutionary changes result in new species. A single lineage may change through time without giving rise to a new species. Although Charles Darwin entitled his book *The Origin of Species*, he did not discuss how a single species splits into two or more daughter species. Rather, he was concerned principally with demonstrating that species are altered by natural selection over time.

The critical process in the formation of new species is the separation of the gene pool of the ancestral species into two separate gene pools. Subsequently, within each isolated gene pool, allele and gene frequencies may change as a result of the action of evolutionary agents. If sufficient differences accumulate during this period of isolation, the two populations may not exchange genes if they come together again.

Gene flow among populations may be interrupted in several ways, each of which characterizes a mode of speciation. The next three sections focus on these modes of speciation: allopatric speciation, sympatric speciation, and parapatric speciation.

Increasing

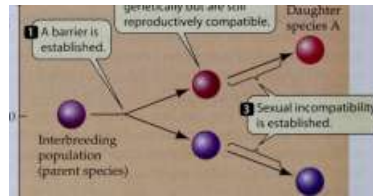
u

o)

c

Increasing

fj Populations diverge genetically but are still reproductively compatible.



Interbreeding population (parent species)

Daughter species B

Time

22.2 Speciation May Be a Gradual Process

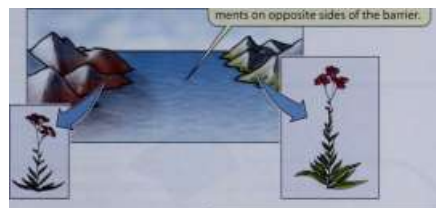
In this hypothetical example, genetic divergence begins before reproductive incompatibility evolves.

Time

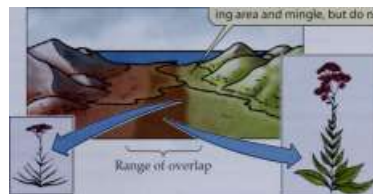
A single species is distributed over a broad range.



Sea level rises and isolates species. Populations adapt to differing environments on opposite sides of the barrier.



If the barrier to breeding is removed, the populations may recolonize the intervening area and mingle, but do not interbreed.



SPECIES AND THEIR FORMATION 415

22.3 Allopatric Speciation

Also known as geographic speciation, allopatric speciation may result when a population is divided into two separate populations by a physical barrier such as rising seas.

suit of new populations founded by individuals dispersing among the islands, because the closest relative of a species on one island is often a species on a neighboring island, rather than a species on the same island. Biologists who have studied the chromosomes of picture-winged *Drosophila* believe that speciation among these flies has resulted from at least 45 such founder events (Figure 22.4).

The finches of the Galapagos archipelago, 1,000 km off the coast of Ecuador, demonstrate the importance of geographic isolation for speciation. Darwin's finches (as they are usually called, because Darwin was the first scientist to study them)

arose on the Galapagos by speciation from a single South American species that colonized the islands. Today there are 14 species of Galapagos finches, all of which differ strikingly from the blue-black grassquit, their probable mainland ancestor (Figure 22.5).

The islands of the Galapagos archipelago are sufficiently isolated from one another that finches

Picture-winged

Drosophila

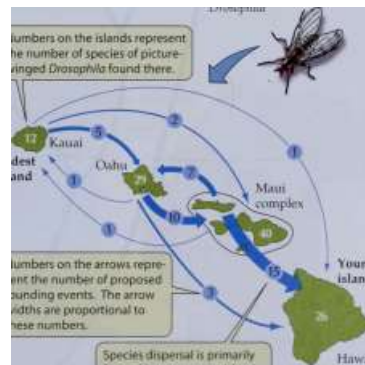
Numbers on the islands represent the number of species of picture-winged *Drosophila* found there.

Allopatric speciation requires total genetic isolation

Speciation that results when a population is isolated by a geographic barrier is known as allopatric speciation (allo-, "different"; patri-, "country"), or geographic speciation (Figure 22.3). Allopatric speciation is thought to be the most common form of speciation among most groups of organisms. The range of a species may be divided by a barrier such as a water gap for terrestrial organisms, dry land for aquatic organisms, or a mountain range. Barriers can form when continents drift, sea levels rise, or climates change. Populations separated in this way are often large initially. They evolve differences because the places in which they live are, or become, different.

Alternatively, allopatric speciation may result when some members of a population cross an existing barrier and found a new population. Populations established in this way usually differ genetically from their parent populations because a small group of founding individuals has only an incomplete representation of the genes found in its parent population (see Chapter 21). Many of the hundreds of species of the fruit fly *Drosophila* in the Hawaiian Islands are restricted to a single island. They are almost certainly the result of

Oldest island

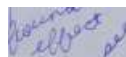


Numbers on the arrows represent the number of proposed founding events. The arrow widths are proportional to these numbers.

Youngest

Species dispersal is primarily from older to younger islands.

Hawaii



22.4 Founder Events Lead to Allopatric Speciation

The extremely high level of speciation found among picture-winged *Drosophila* in the Hawaiian Islands is almost certainly the result of founder events—new populations founded by individuals dispersing among the islands. The islands, which were formed in sequence as Earth's crust moved over a volcanic "hot spot," vary in age.

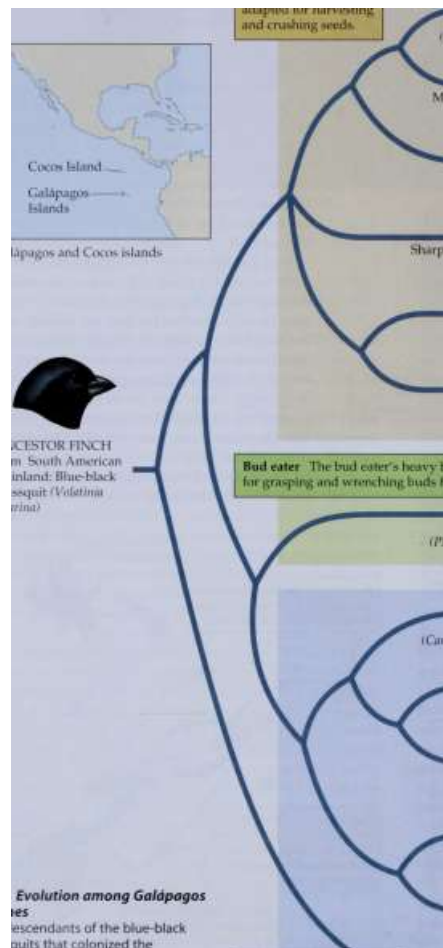
416 CHAPTER TWENTY-TWO

Seed eaters

Bills of seed eaters are adapted for harvesting and crushing seeds.

Galapagos and Cocos islands

Sharp-billed ground finch (*G. difficilis*)



Large ground finch (*Geospiza magnirostris*)

Medium ground finch

(*G. fortis*)

Small ground finch

(*G. fuliginosa*)

Large cactus finch (*G. conirostris*)

Cactus finch (*G. scandens*)

ANCESTOR FINCH from South American mainland: Blue-black grassquit (*Volatinia jacarina*)

Bud eater The bud eater's heavy bill is adapted for grasping and wrenching buds from branches.

22.5 Evolution among Galapagos Finches

The descendants of the blue-black grassquits that colonized the Galapagos archipelago several million years ago evolved into 14 species whose members are variously adapted to feed on seeds, buds, and insects. (The fourteenth species, not shown here, lives on Cocos Island, farther north in the Pacific Ocean.)

Insect eaters

The bills of insect eaters vary because they eat different types and sizes of insects and they capture them in different ways.

Vegetarian finch

(*Platyspiza crassirostris*)

Small tree finch (*Camarhynchus parvulus*)

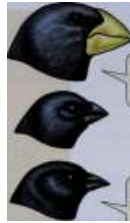
Large tree finch (*C. psittacula*)

Medium tree finch (*C. pauper*)

Mangrove finch (*C. heliobates*)

Woodpecker finch (*C. pallidus*)

Warbler finch (*Certhidca olivacea*)



Large-billed finches can crush large, hard seeds.



Small-billed finches cannot crush large seeds as well, but they are more adept at handling small seeds.

Cactus finches are adapted to opening cactus fruits and extracting the seeds.

^fc



The large tree finch uses its heavy bill to twist apart wood to reach larvae inside.

The small and medium tree finches and mangrove finch pick insects from leaves and branches and explore crevices for hidden prey.

The woodpecker finch uses its long beak to probe dead wood, crevices, and bark for insects.

The warbler finch uses quick motions to capture insects on plant surfaces.

seldom migrate between them. Also, environmental conditions differ among the islands. Some are relatively flat and arid; others have forested mountain slopes. Populations of finches on different islands have differentiated enough that when occasional immigrants arrive from other islands, they either do not breed with the residents, or if they do, the resulting offspring do not survive as well as those produced by pairs of island residents. The genetic distinctness and cohesiveness of the population is thus maintained.

A barrier's effectiveness at preventing gene flow depends on the size and mobility of the species in question. What is an impenetrable barrier to a terrestrial snail may be no barrier at all to a butterfly or a bird. Populations of wind-pollinated plants are isolated at the maximum distance their pollen is blown by the wind, but individual plants are effectively isolated at much shorter distances. Among animal-pollinated plants, the width of the barrier is the distance that animals travel while carrying pollen or seeds. Even animals with great powers of dispersal are often reluctant to cross narrow strips of unsuitable habitat. For animals that cannot swim or fly, narrow water-filled gaps may be effective barriers.

Indirect evidence that most speciation among animals is allopatric is provided by patterns of species distributions. For example, 36 percent of Earth's 20,000 species of bony fishes live in fresh water, even though only 1 percent of Earth's surface is fresh water, and even though fish productivity and populations are higher in some marine environments than in most fresh waters. Because they are highly fragmented, fresh waters have provided abundant opportunities for fishes to form geographically isolated populations. Marine environments provide fewer such opportunities.

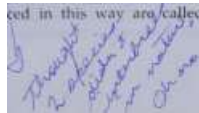
Sympatric speciation occurs without physical separation

The ciihHjviHing of a gnpn pool whpn members of the daughter species are not geographically separated is called

s ympa tric speciation] (sym-, "with"). The most common means of sympatric speciation is polyploidy , anincrease in the number of chromosomes.

Polyploidy arises in two ways. One way is the accidental production during cell division of cells having four (tetra-ploid) instead of tw o (dipl oid) sets of chromosomes. This process produces an autopolyploid individual, one having more than two sets of chromosomes, all derived from a sin-gle _species. This tetraploid individual cannot produce viable offspring by mating with diploids, but it can do so if it s elf-fertilizes or mates with other tetraploid s.

A polyploid species can also be produced when indiv idu als of two different species interbree d. The resulting offsprin g are usually ste rile, because the chromosomes from one species dojiot pair properly with those from the other species during meiosis, but they may be able to reprodu ce a sgxual ly. After many generations, some of these individuals may become fertile as a result of further chromosome duplication. Species produced in this way are >olypToi(

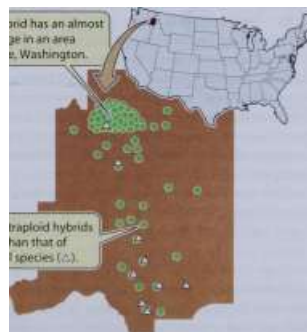


Polyploidy can create new species among ^plants^ much more easily than among animals because plants of many species can re produce by s elf-fertilization. If polyploidy arises in several offspring of a single parent, the siblings can fertilize one another. Speciation by polyploidy has been very important in the evolution of flowering plants. Botanists estimate that about 70 percent of floweri ng plant s pecies and 95 p ercent of all fern species are polyploid s.^*-^ Most of these arose as a result of hy ktfdiy ation hpfwppn e>J ^' t wo species^iollowpd by self-fertilizati on.

The speed with which allopolyploidy can produce new species is illustrated by salsifies (Tragopogon), members of the sunflower family. Salsifies are weedy plants that thrive in disturbed areas around towns. People have inadvertently spread them around the world from their ancestral ranges in Eurasia. Three diploid species of salsify were introduced into North America early in the twentieth century: *T. porrifolius*, *T. pratensis*, and *T. diibius*. Two tetraploid hybrids— *T. minis* and *T. miscellus* —between species of the original three were first reported in 1950. Both hybrids have spread since their discovery and today are more widespread than their diploid parents (Figure 22.6).

Studies of their genetic material have shown that both hybri ds have been formed more than once. Some populations of *T. miscellus* —a hybrid of *T. pmtensis* and *T. dubius* — have the chloroplast genome of *T. pratensis*, whereas other populations have the chloroplast genome of *T. dubius*. Such differences among local populations of *T. miscellus* show that this allopolyploid has evolved independently at least 2 1 times; *T. minis* has formed 12 times ! Scientists seldom know the dates and locations of species formation so well.

A tetraploid hybrid has an almost continuous range in an area around Spokane, Washington.



The range of tetraploid hybrids J () is broader than that of diploid parental species (A).

22.6 Polyploids Can Outperform Their Parents

Tragopogon species (salsifies) are members of the sunflower family. The map shows the distribution of the diploid parent species and the tetraploid hybrid species of Tragopogon in eastern Washington and adjacent Idaho.

418 CHAPTER TWENTY-TWO

The success of newly formed hybrid species of salsifies illustrates why so many species of flowering plants originated as polyploids.

Among animals, sympatric speciation apparently is rare, but may result from precise selection of habitat and mating sites by individuals. A good example is speciation in a picture-winged fruit fly (*Rhagoletis pomonella*) in New York State. Until the mid-1800s, these fruit flies courted, mated, and deposited their eggs only on hawthorn fruits. The larvae learned the odor of hawthorn as they fed on the fruits, and when they emerged from their pupae, they used this food-based memory to locate other hawthorn plants on which to mate and lay eggs.

About 150 years ago, large commercial apple orchards were planted in the Hudson River Valley. Apple trees are closely related to hawthorns, and a few female *Rhagoletis* laid their eggs on apples, perhaps by mistake. Their larvae did not grow as well as larvae on hawthorn berries, but ma ny did su rvive. These larvae had learned the odor of apples, so when they

emerged as adults they sought out apple trees, where they mated with other apple-reared-on-apples.

Today there are two sympatric species of *Rhagoletis* in the Hudson River Valley. One feeds on hawthorn fruits, the other on apples. The two species are reproductively isolated because they mate only with individuals raised on the same fruit, and because they emerge from their pupae at different times. In addition, apple-feeding flies have evolved so that they now grow more rapidly on apples than they originally did.

Parapatric speciation separates adjacent populations

Sometimes reproductive isolation develops between adjacent populations in the absence of a geographic barrier. This type of speciation, known as parapatric speciation (para-, "beside"), is, in effect, all opatric speciation in which the barrier is not a physical barrier, but a difference in conditions. For parapatric speciation to happen, natural selection must be strong enough to overcome gene flow; otherwise, gene flow would prevent differentiation between the two populations. Thus, any factor that reduces gene flow or increases the gradient in selective pressures across small distances can generate conditions favorable for parapatric speciation.

Both kinds of factors are provided by the abrupt changes in soil composition that are created by mining activities that leave rubble (tailings) with high concentrations of heavy metals, such as lead and zinc. Soils that develop on these tailings contain concentrations of heavy metals that are detrimental to the growth of most plants. There is strong selection for heavy metal tolerance in plants growing on the tailings, and within the last several centuries plants able to grow on such soils have evolved in several species of grasses. *Anthoxanthum odoratum* is one of these species. Nearly complete reproductive isolation exists between *A. odoratum* plants growing on tailings and those growing on normal soil because they flower at different times. In addition,

metal-tolerant plants self-pollinate more frequently than plants growing on normal soil, further reducing gene flow. Reproductive isolation between metal-tolerant and metal-intolerant plants is almost complete, demonstrating that gene flow can slow or stop even in the absence of a distinct physical barrier.

It is difficult to determine the importance of parapatric speciation in nature because species ranges change over time. Thus, species with adjacent ranges could have arisen parapatrically where their ranges now come into contact, or they could have arisen in geographic isolation and subsequently expanded their ranges. For this reason, parapatric speciation could be more common than it is generally believed to be.

Reproductive Isolating Mechanisms

Once a barrier to gene flow is established, by whatever means, the resulting daughter populations may diverge genetically because of the action of evolutionary agents. Over many generations, differences that reduce the probability of members of the two populations mating and producing viable offspring may accumulate. In this way, reproductive isolation can evolve as an incidental by-product of other genetic changes in daughter populations. For example, individuals in the two daughter populations may become so different that they are not recognized as suitable mates.

However, geographic isolation does not necessarily lead to reproductive incompatibility. For example, American and European sycamores have been physically isolated from one another for at least 20 million years. Nevertheless, they are morphologically very similar (Figure 22.7), and they can form fertile hybrids. They lack traits that would prevent individuals of the two different populations from producing fertile hybrids. In this section we examine the



(a) *Plantanus occidentalis* (American sycamore)

(b) *Platamis hispanica* (European sycamore)

22.7 Geographically Separated, Morphologically Similar

Although they have been separated on different continents for at least 20 million years, American and European sycamores have diverged very little in appearance.

ways in which such traits— reproductive isolating mechanisms —arise. Then we explore what happens when reproductive isolation is incomplete.

Prezygotic barriers operate before mating

Reproductive isolating mechanisms that operate before mating— prezygotic reproductive barriers —may prevent individuals of different species from interbreeding.

► Spatial isolation. Individuals of different species may select different places in the environment in which to live. As a result,

they may never come into contact during their respective mating seasons; that is, they are reproductively isolated by location.

- Temporal isolation. Many organisms have mating periods that are as short as a few hours or days. If the mating periods of two species do not overlap, they will be reproductively isolated by time.
- Mechanical isolation. Differences in the sizes and shapes of reproductive organs may prevent the union of gametes from different species.
- Gametic isolation. Sperm of one species may not be attracted to the eggs of another species because the eggs do not release the appropriate attractive chemicals, or the sperm may be unable to penetrate the egg because it is chemically incompatible.

Postzygotic barriers operate after mating

If individuals of two different species still recognize one another and mate, postzygotic reproductive barriers may prevent gene exchange. Accumulated genetic differences are likely to reduce the fitness of offspring produced by matings between individuals from the two species.

- Hybrid zygote abnormality. Hybrid zygotes may fail to mature normally, either dying during development or developing such severe abnormalities that they cannot mate.
- Hybrid infertility. Hybrids may mature normally, but be infertile when they attempt to reproduce. For example, the offspring of matings between horses and donkeys—mules—are vigorous, but sterile; they produce no descendants (Figure 22.8).
- Low hybrid viability. Hybrid offspring may survive less well than offspring resulting from matings within each species.
- Absence or sterility of one sex. In nearly all cases of hybrid sterility and hybrid inviability, it is the sex that is heterozygous for the sex chromosomes (XY, XO, or ZW; see Chapter 10) that is absent or sterile. The reason is that any deleterious recessive alleles on a sex chromosome are fully expressed in hybrids of the sex with only one copy.

If hybrid offspring survive or reproduce poorly, postzygotic barriers may be reinforced by the evolution of more effective prezygotic barriers. More effective prezygotic barriers should evolve if individuals engaging in hybrid matings leave fewer surviving offspring than individuals that mate only within their own species. Reinforcement of prezygotic barriers has been demonstrated in a few laboratory populations, but evidence for it in nature has been slow to accumulate.



22.8 Sturdy but Sterile

Mules are widely used as pack animals because of their stamina. For that purpose, their infertility is unimportant.

Sometimes reproductive isolation is incomplete

If contact is reestablished between two formerly geographically isolated populations before many genetic differences have accumulated, the two populations may interbreed freely with each other, and their hybrid offspring may be as successful as those resulting from matings within each population. If hybrids spread through both populations and reproduce with other individuals, the gene pools combine quickly, and no new species results from the period of isolation. Alternatively, the two populations may interbreed only where they come into contact, resulting in a hybrid zone.

Detailed studies are being carried out in a narrow zone in Washington where Townsend's warblers and hermit warblers hybridize (Figure 22.9). Both of these warblers breed in tall conifer forests, and no habitat boundaries exist at the locations of the hybrid zones. The zone is narrow because of natural selection against hybrids. It is shifting southward because Townsend's warblers are replacing hermit warblers. Townsend's males are more aggressive than hermit males toward stuffed males of the other species placed in their territories, and they are better at attracting mates than hermit warbler males.

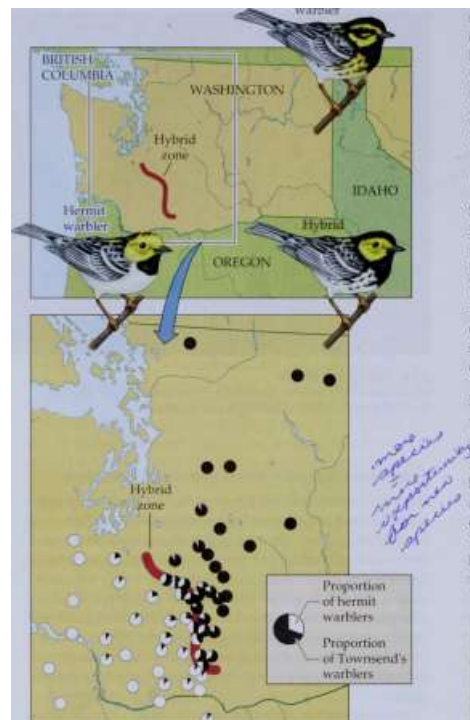
Species may differ in relatively few genes

If two species hybridize, we know that they are similar genetically. The absence of interbreeding, however, tells us nothing about how dissimilar two species are. Not until modern molecular techniques were developed could biologists measure genetic differences among species.

Molecular studies are now demonstrating that many sympatric species may be genetically very similar to one another. For example, different species of Hawaiian *Drosophila*

420 CHAPTER TWENTY-TWO

[bwnsend's warbler



100



Townsend's warblers competed for and maintained breeding territories better than hermit warbler rivals...

...and hermit warblers were more likely to be replaced in territories or abandon them than either Townsend's or hybrids.

J]

| = Townsend's 1 = hybrids] = hermits

Maintained territory

Replaced in territory

Abandoned territory

22.9 Hybrid Zones May Shift over Time

The zone where Townsend's and hermit warblers hybridize is shifting to the south because male Townsend's warblers dominate male hermit warblers.

share nearly all of their mitochondrial DNA alleles. However, only a small fraction of the genes of these species have been analyzed, so genetic differences may be greater than we now think. All of the hundreds of species of *Drosophila* that have evolved in Hawaii during the past 32 million years, even those that have diverged morphologically, are relatively similar genetically (Figure 22.10).

Variation in Speciation Rates <

Some lineages of organisms contain many species; others have only a few. Hundreds of species of *Drosophila* evolved in the Hawaiian Islands, but there is only one species of horseshoe crab, even though its lineage has survived more than 200 million years. Why do rates of speciation vary so widely among lineages? In the sections that follow we will examine several factors that influence speciation rates: species richness, range size, behavior, environmental changes, and generation times.

Species richness may favor speciation

The larger the number of species there are in a lineage, the larger the number of opportunities for new species to form. This is particularly true of speciation by polyploidy because more species are available to hybridize with one another. It is also partly true of allopatric speciation, because the larger the number of species living in an area, the larger the number of species whose ranges will be bisected by a given barrier.



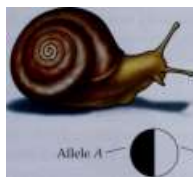
Drosophila conspurcator

Drosophila obscura

22.10 Morphologically Different, Genetically Similar

Although these fruit flies—a small sample of the hundreds of species found only on the Hawaiian Islands—are extremely variable in appearance, they are genetically similar.

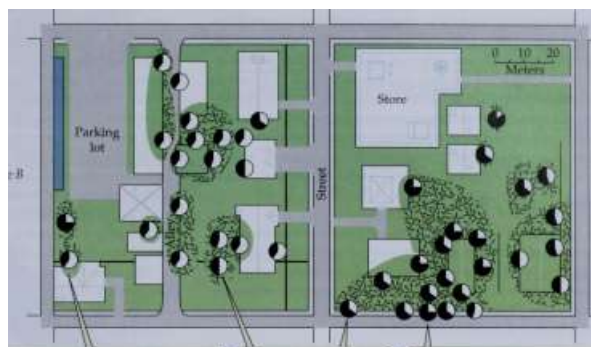
Helix aspersa



Allele A

Allele B

22.11 Mobility Affects Speciation Rates Even a narrow street, such as this one in a Texas study, presents a geographic barrier to land snails. Because snails rarely cross city streets, populations on opposite sides of a street may differ strikingly genetically.



Each circle represents a small colony of snails, *Helix aspersa*, living in a patch of vegetation adjacent to buildings.

Allele frequencies differ on opposite sides of the street.

Note the dramatic increase in allele A on the right side of the street.

Range size may affect speciation rates

Relationships between range size and speciation rate are not simple, however, because the ranges of individual species tend to be small where there are many species. The larger the range of a species, the more likely a physical barrier is to subdivide it. Also, species with large ranges are more likely than species with small ranges to establish isolated peripheral populations that survive long enough to form new species.

Behavior may influence speciation rates

The mobility of a species may influence how often its range is likely to be divided by barriers. Individuals of species with poor dispersal abilities are unlikely to establish new populations by dispersing across barriers, and even narrow barriers effectively isolate species whose individuals are highly sedentary. Populations of land snails may be separated by barriers as narrow as city streets (Figure 22.11).

Animals with complex behavior are likely to speciate at a high rate because they make sophisticated discriminations among potential mating partners. They distinguish members of their own species from members of other species, and they make subtle discriminations among members of their own species on the basis of size, shape, appearance, and behavior. Such discrimination can greatly influence which individuals are most successful in producing offspring. Therefore, mate selection is a cause of rapid evolution of reproductive isolation between species.

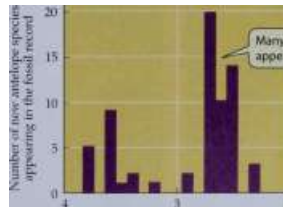
Environmental changes may trigger high speciation rates

African antelopes underwent a burst of speciation and extinction between 2.5 and 2.9 million years ago. During that period, the number of known antelope species doubled,

and 90 percent of all antelope species known to have existed at that time either first appeared or went extinct (Figure 22.12). This burst coincided with a shift in Africa from a warm and wet climate to one that oscillated between warm, wet and cooler, drier conditions. The burst of speciation among antelopes resulted in many more species adapted to grasslands and savannas, environments that increased and decreased, thereby coalescing and separating over much of Africa, as the climate oscillated.

Short generation times enhance speciation

We have been concentrating on factors that influence rates at which the ranges of species are subdivided by barriers.



Many new species appeared 2.5-2.9 mya.

11

32]

Millions of years ago

22.12 Climate Change Drove a Burst of Speciation among Antelopes

The excellent fossil record of African antelopes reveals that there was a sudden burst of speciation between 2.5 and 2.9 million years ago. At that time, the climate of Africa shifted from being consistently warm and wet to oscillating between warm and wet and cool and dry.

422 CHAPTER TWENTY-TWO

But the rate at which new species form also depends on how fast daughter populations diverge. The more rapidly they diverge, the sooner they are likely to evolve reproductive isolating mechanisms, and the less likely they are to hybridize if they again become sympatric. Shorter generation times result in more generations per unit of time and, as a result, generate the potential for more evolutionary changes per unit of time.



Evolutionary Radiations

As we learned in Chapter 20, the fossil record reveals that at certain times in some lineages, speciation rates have been much higher than extinction rates. The result is an evolutionary radiation that gives rise to a large number of daughter species. What conditions cause speciation rates to be much higher than extinction rates?

Evolutionary radiations are likely when a population colonizes an environment that has relatively few species. This condition typifies island life because many organisms disperse poorly across large water gaps. Because islands lack many plant and animal groups found on the mainland, ecological opportunities exist that may stimulate rapid evolutionary changes when a species does reach them. Water barriers also restrict gene flow among islands in an archipelago, so populations on different islands can evolve adaptations to their local environments. Together these two factors make it likely that speciation rates on island archipelagos will exceed extinction rates.

Remarkable evolutionary radiations have occurred in the Hawaiian Islands, the most isolated islands in the world. The Hawaiian Islands lie 4,000 km from the nearest major land mass and 1,600 km from the nearest group of islands. The islands are arranged in a line of decreasing



age—the youngest islands in the southeast, the oldest to the northwest. The archipelago is actually much older than the oldest existing islands because even older islands long ago eroded until they no longer rise above the sea surface.

The native biota of the Hawaiian Islands includes 1,000 species of flowering plants, 10,000 species of insects, 1,000 land snails, and more than 100 bird species. However, there are no amphibians, no terrestrial reptiles, and only one native mammal—a bat—until humans introduced additional species.

The 10,000 known native species of insects on Hawaii are believed to have evolved from only about 400 immigrant species; only 7 immigrant species are believed to account for all the native Hawaiian land birds.

More than 90 percent of all plant species on the Hawaiian Islands are endemic—that is, they are found nowhere else. Several groups of flowering plants have more diverse forms and life histories on the islands and live in a wider variety of habitats than do their close relatives on the mainland. An outstanding example is the group of sunflowers (silverswords) (the genera *Argyroxiphium*, *Dubautia*, and *Wilkesia*). Chloroplast DNA data show that these species share a relatively recent common ancestor, which is believed to be a species of tarweed from the Pacific coast of North America. Whereas all mainland tarweeds are small, upright, nonwoody plants (herbs), Hawaiian silversword species include prostrate and upright herbs, shrubs, trees, and vines (Figure 22.13). They occupy nearly all the habitats of the islands, from sea level to above timberline in the

22.13 Rapid Evolution among Hawaiian Plants

Three closely related genera of the sunflower family, two of which are illustrated here, are believed to have descended from a single ancestor, a tarweed (*Madia sativa*) that colonized Hawaii from the Pacific coast of North America. They appear more distantly related than they actually are.

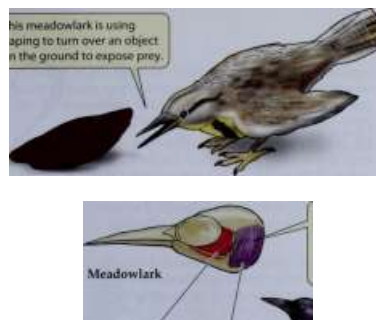


Madia sativa (ancestral tarweed)

Argyroxiphium sandwichense

Dubautia menziesii

This meadowlark is using gaping to turn over an object on the ground to expose prey.



The powerful gaping muscles of the meadowlark, one of the American blackbirds, enable it to expose prey buried in soil or under objects.

Crushing Gaping muscles muscles



Common grackle

Because the common grackle has weak gaping muscles, it must feed on exposed prey and crush its food.

22.14 Blackbirds Expose Food by Gaping

The species of blackbirds that find food by gaping, here illustrated by a meadowlark, differ strikingly in the size and strength of their gaping muscles from blackbird species (such as grackles) that feed on exposed food.

mountains. Despite their extraordinary diversification, however, the silverswords have differentiated very little in their chloroplast genes.

The island silverswords are more diverse in size and shape than the mainland tarweeds because the original colonizers arrived on islands that had very few plant species. In particular, there were few trees and shrubs, because such large-seeded plants rarely disperse to oceanic islands.

Many island trees and shrubs have woody ancestors.

On the mainland, however, tarweeds live in ecological communities that contain tree and shrub lineages older than their own—that is, where opportunities to exploit the tree way of life were already preempted.

Evolutionary lineages may also radiate when they acquire a new adaptation that enables them to use the environment in new and varied ways. For example, ancestors of the 95 species of American blackbirds evolved powerful muscles for opening their bills. These muscles enable the birds to obtain food by opening their bills forcibly against objects they wish to move, exposing otherwise hidden prey (Figure 22.14). This behavior is called *gaping*. Birds lacking these powerful muscles can find prey only on exposed surfaces of objects. Blackbirds gape into wood, fruits, leaf clusters, and stems of nonwoody plants; under sticks, stones, and animal droppings; and into the soil. With this feeding method, they have come to occupy nearly all habitat types

SPECIES AND THEIR FORMATION 423

in North and South America, and they are among the most abundant birds throughout the region.

The Significance of Speciation

The result of speciation processes operating over billions of years is a world in which life is organized into millions of species, each adapted to live in a particular environment and to use environmental resources in a particular way. Earth would be very different if speciation had been a rare event in the history of life. How the millions of species are distributed over the surface of Earth and organized into ecological communities will be a major focus of Part Seven of this book, "Ecology and Biogeography." There we will also discuss how human activities are causing the extinction of species and what we can do to reduce the rate of species loss.



Chapter Summary

What Are Species?

- Species are independent evolutionary units. A commonly accepted definition is that "species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups."
- Because speciation is often a gradual process, it may be difficult to recognize boundaries between species. Review Figure 22.2

How Do New Species Arise?

- Not all evolutionary changes result in new species.
- Allopatric (geographic) speciation is the most important means of speciation among animals and is common in other groups of organisms. Review Figures 22.3, 22.4, 22.5
- Species may form sympatrically by a multiplication of chromosome numbers because the resulting polyploid organisms cannot interbreed with members of the parent species. Polyploidy has been a major factor in plant speciation, but is rare among animals. Review Figure 22.6
- Species may form parapatrically where marked environmental differences prevent gene flow among individuals living in adjacent environments.

Reproductive Isolating Mechanisms

- When previously allopatric species become sympatric, reproductive isolating mechanisms may prevent the exchange of genes.
- Barriers to gene exchange may operate before mating (prezygotic barriers) or after mating (postzygotic barriers).
- Hybrid zones may develop if barriers to gene exchange failed to develop during allopatry.

► Hybrids may form if separated populations come together again without sufficient genetic differences having accumulated. Review Figure 22.9

► The existence of hybrids tells us that the two hybridizing species are very similar genetically, but species that do not hybridize may also differ from one another very little genetically.

Variation in Speciation Rates

► Rates of speciation differ greatly among lineages of organisms. Speciation rates are influenced by the number of

424 CHAPTER TWENTY-TWO

species in a lineage, their range sizes, their behavior, environmental changes, and generation times. Review Figures 22.11, 22.12

Evolutionary Radiations

► Evolutionary radiations happen when speciation rates exceed extinction rates.

► High speciation rates often coincide with low extinction rates when species invade islands that have few other species, or when a new way of exploiting the environment makes a different array of resources available to a species. Review Figures 22.13, 22.14

The Significance of Speciation

► As a result of speciation, Earth is populated with millions of species, each adapted to live in a particular environment and to use resources in a particular way.

For Discussion

1. Gene exchange between populations is prevented by geographical isolation, by behavioral responses before mating (for example, females may reject courting males of the other species), and by mechanisms that function after mating has occurred (for example, hybrid sterility). All of these are commonly called isolating mechanisms. In what ways are the three types very different? If you were to apply different names to them, which one would you call an isolating mechanism? Why? What names would you give the other types? Why?

2. The blue goose of North America has two distinct color forms, blue and white. Matings between the two color types are common. However, blue individuals pair with blue individuals and white individuals pair with white individuals much more frequently than would be expected by chance. Suppose that 75 percent of all mated pairs

consisted of two individuals of the same color. What would you conclude about speciation processes in these geese? If 95 percent of pairs were the same color? If 100 percent of pairs were the same color?

3. Suppose pairs of blue geese of mixed colors were found only in a narrow zone within the broad Arctic breeding range of the geese, would you answer Question 2 the same way you did? Would your answer change if mixed-color pairs were widely distributed across the breeding range of the geese?

4. Although many species of butterflies are divided into local populations among which there is little gene flow, these butterflies often show relatively little geographic variation. Describe studies you would conduct to determine what maintains this morphological similarity?

5. Distinguish among allopatric, parapatric, and sympatric speciation. For each of the three statements below, indicate which type of speciation is implied:

a. This process in nature is most commonly a result of polyploidy.

b. The size of national parks and wildlife refuges may be too small to allow this type of speciation among organisms restricted to those areas.

c. This process usually occurs in species that inhabit areas where sharp environmental contrasts exist.

6. Evolutionary radiations are common and easily studied on oceanic islands. In what types of mainland situations would you expect to find major evolutionary radiations? Why?

7. Fruit flies of the genus *Drosophila* are found worldwide, but most of the species in the genus are found on the Hawaiian Islands. Suggest a hypothesis that might

' account for this distribution pattern.



Schistosomiasis is a blood infection caused by a parasitic flatworm, *Schistosoma*. More than 200 million people in South America, Africa, China, Japan, and Southeast Asia have the disease. During part of its life cycle, *Schistosoma* inhabits a freshwater snail. People become infected when they come into contact with water where infested snails live. Larval *Schistosoma* swim from a snail and penetrate the skin. The worm matures and lives in the person's abdominal blood vessels. The disease is progressively debilitating, causing a slow death.

For most of the twentieth century, only one species, *Schistosoma japonicum*, was known to infect humans, and people believed that it was transmitted by a single species of snail in the genus *Oncomelania*. Then, in the 1970s, researchers discovered that a different snail was transmitting *Schistosoma* to humans in the Mekong River in Laos. This discovery stimulated extensive field surveys and anatomical, genetic, and geographic research on the worms and snails of Southeast Asia.

Investigators found that *S. japonicum* was actually a cluster of at least six species. They also discovered that evolutionary relationships among snails influenced which species could host *Schistosoma*. Evolutionary diversification from an ancestral stock of snails produced a group of species of modern snails. Of these, only three can host *Schistosoma*; ten have a genetic trait that allows them to resist invasion by the parasite.

This information is of great value in efforts to combat schistosomiasis. Few of the freshwater snail species in Southeast Asia have been described and named. By using information on evolutionary relationships among snails, scientists can quickly determine whether or not a newly discovered snail is likely to be a host for *Schistosoma*. Control efforts need to be directed toward only the snails that can transmit *Schistosoma* to humans, not all freshwater snails in the region.

How did investigators determine the evolutionary relationships among the snails that are hosts of *Schistosoma*? How could they determine the number of times that genes preventing snails from hosting *Schistosoma*

Asian Snails Can Transmit Schistosomiasis

Workers in the rice paddies of tropical Asia are at extreme risk of contracting schistosomiasis (known in some parts of the world as bilharzia). The disease is transmitted to humans via freshwater snails that thrive in the standing water of the paddies.

How is knowledge of evolutionary relationships used to help answer other biological questions? How are evolutionary relationships expressed in systems of classification that help guide further studies of organisms?

In this chapter, we discuss systematics, the science that provides answers to these questions. We describe the methods systematists use to infer evolutionary relationships among organisms. Then we illustrate how knowledge of evolutionary relationships is used to solve other biological problems. Finally, we show how evolutionary relationships are incorporated into classification systems.

How Are Phylogenetic Trees Reconstructed?

Ever since its origin nearly 4 billion years ago, life has evolved under the influence of the evolutionary agents we described in Chapter 21. The incredible richness of today's biological world has resulted from millions of speciation events, determined by the processes we discussed in Chapter 22. Biologists have developed methods to trace the history of these processes and make sense of their results.

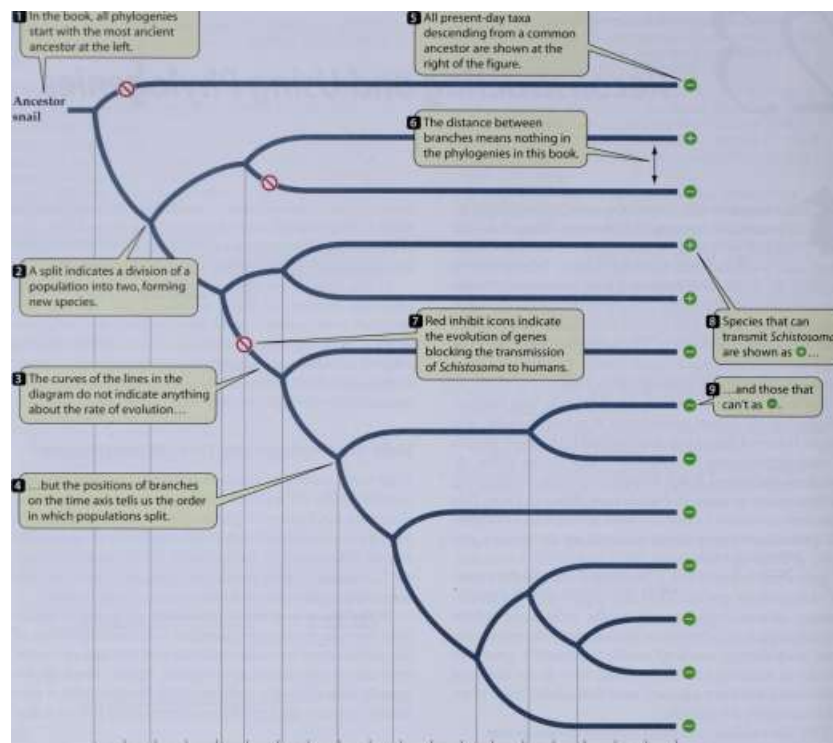
A phylogeny is a history of descent of a group of organisms from their common ancestor. Our understanding of the processes of speciation tells us that lineages of organisms can be represented as branching "trees." These phylogenetic trees show the order in which lineages split. A particular tree may portray the evolution of all life, of major



426 CHAPTER TWENTY-THREE

In the book, all phylogenies start with the most ancient ancestor at the left.

All present-day taxa descending from a common ancestor are shown at the right of the figure.



Most M

ancient

23.1 How to Read a Phylogenetic Tree

A phylogenetic tree displays the order in which lineages split. This example shows the phylogeny of *Oncomelania* snails, the intermediate hosts of the human parasite *Schistosoma*.

evolutionary lineages, or of only a small group of organisms, such as the snail genus *Oncomelania* (Figure 23.1). In the phylogenetic trees in this book, time flows from left (earliest) to right (most recent). It is equally common practice to draw trees with the earliest times at the bottom.

Determining the evolutionary relationships among organisms is intrinsically exciting. We are especially interested in the origin of our own species, but we also care about, for example, the origins of birds and mammals from reptilian ancestors. In addition, phylogenetic information helps us deal with practical problems, such as the control of schistosomiasis. We will return to the uses of phylogeny

■ >- Present

after we have described the methods by which systematists reconstruct phylogenetic trees.

Systematists reconstruct phylogenetic trees by analyzing evolutionary changes in the traits of organisms. Phylogenetic trees are rather like pedigrees, except that they are usually constructed with the ancestor at the base rather than on the "twigs." The base of a phylogeny represents the point in the past when the lineage consisted of only the ancestor.

Charles Darwin described evolution as descent with modification. He recognized that closely related species—that is, species that share a recent common ancestor—are likely to be very similar. In other words, they should share many traits that they inherited from the common ancestor. Systematists expect traits inherited from an ancestor in the dis-

Bat wing



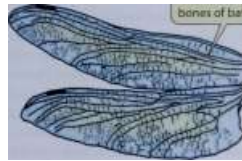
RECONSTRUCTING AND USING PHYLOGENIES 427

Bones shown in the same color are homologous.

Bird wing



Insect wing



The supports for insect wings are not homologous with the bones of bat and bird wings.

23.2 The Bones of the Wings of Bats and Birds Are Homologous, but the Wings Themselves Are Not

The supporting structures of bat and bird wings are derived from a common tetrapod (four-limbed) ancestor, and are thus homologous. The wings themselves, however, evolved independently in the two groups.

J&*

tant past to be shared by a lar ge number of sp ecies. Traits t Kat first appeared i n a more recent ancestor should be shared by fewer species, . But in all cases, the sharing of traits by a group of species indicates that they mayb e descendants of a common ancestor.

Any two features descended from a common a ncestral fe ature jare said t o be homologou s; these features may be anatomical structures, behavior-patterns, nucleotides in a DNA sequence, or any other heritable trait. Traits that are shared by most or all organisms in any lineage being studied are likely to have been inherited relatively unchanged from an ancestor that lived very long ago. For example, all living vertebrates have a vertebral column, and all known fossil ancestral vertebrates also had a vertebral column. The vertebral column is therefore judged to be homologous in all vertebrates.

A trait that differs from its ancestral form is called a dejrived trait, in order to identify how traits have changed during evolution, systema-tists must infer the state of the trait in some ancestor and then determine how it has been

modified in the descendants. Doing so is real evolutionary patterns are complex, generate difficulties:

)t easy because rhree processes

► Independently evolved features subjected to similar s elective pressures may become superficially simila r as a result of convergent evolution. For example, although tHe~5one5T)f lHe wings o f bats and birds are homologous, having been inherited from a common ancestor, the wings themselves are not homologous ^^ because they evolved independently in bats and in birds from the forelimbs of a nonflying ancestor (Figure 23.2). -\

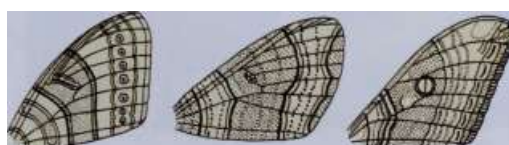
► Similar developmental processes may result in parallel evolution of similar traits in distantly related organisms (Figure 23.3).

► Over time, there may be evolutionary reversals; that is, a character may rpyprt frnm a c\&r\ \ pd_ state back to an a ncestral on e. For example, most frogs lack teeth on their lower jaw, but the ancestors of frogs did have such teeth. One frog genus, *Amphignathodon*, has re-evolved teeth in the lower jaw.

Together these processes generate h omoplastic tra its; that is, traits that are similar for some reason other than inheritance from a common ancestor^

Depending on the size of the lineage we are looking at, a given trait may be ancestral or derived. For example, rats and mice (both rodents), but not dogs or other mammals, have long, c ontinuously growing incisor tee th. Continuously growing incisors evidently developed in the common ancestor of rats and mice after their lineage separated from the one leading-toxLogs and other mammals, because no other mammals have that kind of incisor. Thus, if we were reconstructing a phylogeny of a gro up of rodents, continuously growi ng incisors would be an ance s tral trait beca use "S^* all^ rodentsjTaye_it. However, if we wei£jecoji £tru_cting a ph^lngpnyj^f^jriammak^ growing in rigors

The first step in reconstructing a phylogeny is to select the group of organisms whose phylogeny is to be determined. We will refer to these organisms as th e focalgw up. The next step is to choose the characters that will be used in the analysis and to identify the possible forms (traits) of



Brush-footed butterflies

Inchworm moths

Giant silkworm moths

23.3 Parallel Evolution in Butterfly Wing Bands

Bands on the wings of these distantly related butterflies and moths conform to a common pattern. Similar processes of wing development in all three species produce these similar patterns.

428 CHAPTER TWENTY-THREE

those characters. Recall from Chapter 10 that a character is a feature such as flower color; a trait is a particular form of a character, such as white flowers. A trait may be the presence or absence of a character, or the character may exist in more than one form. The next, and usually the most difficult, step is to determine the ancestral and derived traits. Finally, systematists must distinguish homologous from homoplastic traits.

Identifying ancestral traits

Distinguishing derived traits from ancestral traits may be difficult because traits often become so dissimilar that ancestral states are unrecognizable. For example, the leaves of plants have diverged to form many different structures. Several lines of evidence, especially details of their structure and development, indicate that protective spines, tendrils, and brightly colored structures that attract pollinators (Figure 23.4) are all modified leaves; they are homologous to one another even though they do not resemble one another. One way to distinguish ancestral traits from derived traits is to assume that an ancestral trait should be found not only among the species in the focal group, but also in outgroups. An outgroup

is a lineage that is closely related to the focal group but which branched off from the lineage of the focal group below its base on the evolutionary tree. Traits found only within the focal group, on the other hand, are likely to be derived traits. Species that have a recent common ancestor should share very few homoplastic traits, because little time has been available for convergent evolution to produce them.

The spines of the barrel cactus and the bracts of *Heliconia* are both modified leaves.

Cheiridopsis tuberculata

Heliconia

sp.

The more traits that are measured, the more likely the data will support a single phylogenetic pattern, and the more readily biologists can distinguish between homologies and homoplasies. A few of the traits originally assumed to be homologies may turn out to be homoplasies, but the best way to determine the true status of shared traits is to assume that they are homologous until additional evidence suggests they are not.

rn



Reconstructing a simple phylogeny

To see how a phylogeny is constructed, consider eight vertebrate animals—hagfish, perch, pigeon, chimpanzee, salamander, lizard, mouse, and crocodile. We will assume initially that a given derived trait evolved only once during the evolution of these animals, and that no derived traits were lost from any of the descendant groups. For simplicity, we have selected traits that are either present (+) or absent (-). The traits we will consider are listed in Table 23.1.

As will become evident in Chapter 33, hagfishes are believed to be more distantly related to the other vertebrates than the other vertebrates are to each other. Therefore, we choose hagfishes as the outgroup for our analysis. Derived traits are those that have been acquired by other members of the lineage since they separated from hagfishes.

We begin by noting that the chimpanzee and the mouse share two unique traits, mammary glands and fur. Those traits are absent in both the outgroup and the other species whose relationships we are attempting to determine. Therefore, we infer that mammary glands and fur are derived traits that evolved in a common ancestor of chimpanzees and mice after that lineage separated from the ones leading to the other vertebrates. In other words, we provisionally assume that mammary glands and fur evolved only once among the animals we are classifying.

The pigeon has one unique trait: feathers. As before, we provisionally assume that feathers evolved only once, after the lineage leading to birds separated from that leading to the mouse, chimpanzee, and crocodile. By the same reasoning, we assume that four-chambered hearts evolved only once, after the lineage leading to crocodiles, birds, and mammals separated from the lineage leading to lizards. We assume that claws or nails evolved only once, after the lineage leading to salamanders separated from the lineage leading to those animals that have claws or nails. We make the same assumption for lungs and jaws, continuing to minimize the number of evolutionary events needed to produce the patterns of shared traits among these eight animals.

Using this information, we can reconstruct a provisional phylogeny. The group with no de-

23.4 Homologous Structures Derived from Leaves

The leaves of plants have diverged during their evolution to form many different structures, some of which bear very little resemblance to each other. *Heliconia* bracts support flowers and attract pollinators.

derived traits, the hagfish, is the outgroup, and we assume that the animals that share unique derived traits have a common ancestor not shared with animals lacking those traits. We assume, for exam-

RECONSTRUCTING AND USING PHYLOGENIES 429

Zj. 1 Eight Vertebrates Ordered According to Unique Shared Derived Traits

DERIVED TRAIT a

TAXON

JAWS

LUNGS

CLAWS OR NAILS

FEATHERS

FUR

MAMMARY GLANDS

FOUR-CHAMBERED HEART

' A plus sign indicates the trait is present, a minus sign that it is absent.

pie, that mice and chimpanzees, the only two animals that share fur and mammary glands, share a more recent com-mon ancestor with_ each other than they do with birds and crocodiles. Otherwise we would need to assume that the ancestors of birds and crocodiles also had fur and mammary glands, but that those traits were subsequently lost— unnecessary additional assumptions.

A phvlogeny for these eight vertebrates, based on the traits "we used and the assumption that each derived trait evolved only once, is shown in Figure 23.5. Notice that the phylogeny does not describe the ancestors or date the splits

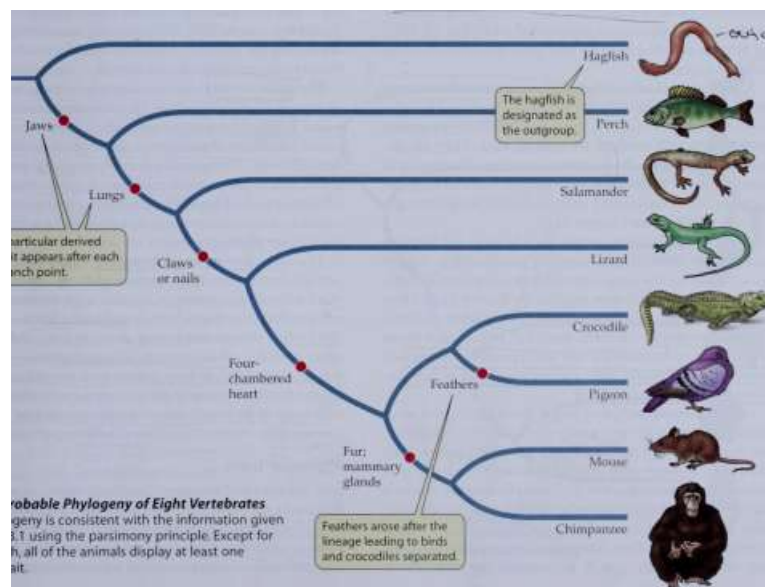
Common ancestor

between lineages. It shows only the sequential order of the splits ^_l'he"o Tdest splits are to th e left, and the moTe recent ones are to the right. Notice also that the y axis hasTTto scale. In this and all other phylogenies in this book, vertical distances between groups do not correlate with degree of similarity or difference between them.

The phylogeny of these eight vertebrates was easy to construct because the traits we chose fulfilled the assumptions that derived traits appeared only once in the lineage and were never lost after they appeared. If we had included a snake in the group, however, our second assumption

'Cx ^sc^S^

A particular derived trait appears after each branch point.



chambered heart

23.5 A Probable Phylogeny of Eight Vertebrates

This phylogeny is consistent with the information given in Table 23.1 using the parsimony principle. Except for the hagfish, all of the animals display at least one derived trait.

Feathers arose after the ineage leading to birds and crocodiles separated.

430 CHAPTER TWENTY-THREE

would have been violated, because the lizard ancestors of snakes had limbs, which were subsequently lost, along with their

claws. We would need to examine additional traits to determine that the lineage leading to snakes separated from the one leading to lizards long after the lineage leading to lizards separated from the others. In fact, the analysis of many traits shows that snakes evolved from burrowing lizards that lost their limbs during a long period of subterranean existence.

Many traits must be analyzed to reconstruct a phylogeny, and systematists use various methods to combine information from the different traits. The simple method we used in our vertebrate example does not work in the vast majority of cases because we know from fossil and other evidence that traits can change more than once, or even undergo reversal. How do systematists deal with these complexities when they reconstruct phylogenies?

The most widely used methods of reconstructing phylogenetic trees employ the parsimony principle. (In its most general form, the parsimony principle states that one should prefer the simplest hypothesis that is capable of explaining the known facts.) The application of the parsimony principle to the reconstruction of phylogenies means minimizing the number of evolutionary changes that need to be assumed over all characters in all groups in the tree—that is, the best hypothesis is the one that requires the fewest homoplasies.

Parsimony works best for morphological traits, whose evolutionary rates are generally slow enough that similarities due to homoplasies are uncommon relative to the number of traits retained because they were inherited from the common ancestor.

Another method, called the maximum likelihood method, is used primarily for the reconstruction of phylogenies based on molecular data. The computer programs employed in this method are complicated. They are designed to deal with the fact that mutations that result in substitutions of nucleotides are common, but that their frequencies can be estimated independently from other genetic information (see Chapter 24).

Using the parsimony principle is helpful not because evolutionary changes are necessarily parsimonious, but because it is generally wiser not to adopt complicated explanations when simpler ones explain the known facts. More complicated explanations are accepted only when evidence requires them. Phylogenetic trees are hypotheses about evolutionary relationships that are repeatedly tested and modified as additional traits are measured and as new fossils

Whatever method is employed, determining the most likely phylogeny for any group of organisms is difficult. For example, there are 34,459,425 possible phylogenetic trees for a lineage with only 11 species! Computer programs using the parsimony principle employ various search routines that calculate the shortest possible phylogenetic tree—that is, with the fewest homoplasies—for a given data set and then compare other possible phylogenies with the shortest one. If, as is usually the case, several

trees are of approximately equal length, they can be merged into a consensus tree that retains only those lineage splits that are found in all the most parsimonious trees. In a consensus tree, groups whose relationships differ among the trees form nodes with more than two branches. These nodes are considered "unresolved" because during speciation, a lineage typically splits into only two daughter species.

Traits Used in Reconstructing Phylogenies

Because organisms differ in many ways, systematists use many traits to reconstruct phylogenies. Some of these traits are readily preserved in fossils; others, such as behavior and molecular structure, rarely survive fossilization processes. Systematists take into consideration behavioral and molecular traits as well as structural traits in both living and fossil organisms. The more traits that are measured, the more inferred phylogenies should converge on one another and on the actual evolutionary pattern.

Morphology and development

An important source of information for systematists is morphology—that is, the sizes and shapes of body parts. Because living organisms have been studied for centuries, we have a wealth of morphological data, as well as extensive museum and herbarium collections of organisms whose traits can be measured. Sophisticated methods are now available for measuring and analyzing morphology and for estimating the amount of morphological variation among individual populations, and species.

The fossil record, which reveals when lineages diverged and began their independent evolutionary histories, can tell us the timing of evolutionary events. Fossils provide important evidence that helps us distinguish ancestral from derived traits. They provide the only available information about where and when organisms lived in the past and what they looked like. When available, this information is valuable, but sometimes few or no fossils have been found for a group whose phylogeny we wish to determine.

The early developmental stages of many organisms reveal similarities to other organisms that are lost by the time of adulthood. For example, the larvae of the marine creatures called sea squirts have a rod-shaped notochord—that disappears as they develop into adults. Many other animals—all chordates—also have this structure at some time during their development. This shared structure is one of the reasons for believing that sea squirts are more closely related to vertebrates than would be suspected by examination of the adults only (Figure 23.6).

Molecular traits

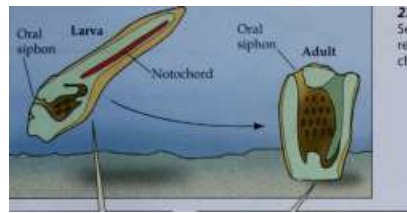
Like the sizes and shapes of their body parts, the molecules that make up organisms are heritable characteristics that may diverge among lineages over evolutionary time. (Molecular evolution will be discussed in detail in Chapter 24. The molecular traits most useful for constructing phylogenies

Sea squirt

(seen in section)

Oral Larva siphon

Adult



The free-swimming, immature form (larva) of the sea squirt and the vertebrate embryo (frog) both have a notochord for body support.

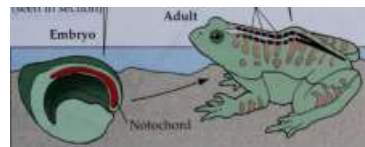
Both the adult form of the sea squirt and the adult frog lack notochords. In the adult frog, the vertebral column replaces the notochord as the support structure.

Frog embryo (seen in section)

Embryo

Adult

Vertebral column



RECONSTRUCTING AND USING PHYLOGENIES 431

23.6 A Larva Reveals Evolutionary Relationships

Sea squirt larvae, but not adults, have a well-developed notochord that reveals their relationship with vertebrates, all of which have a notochord at some time during their life cycle.

myoglobin pseudogene (a nonfunctional DNA sequence derived early in primate evolution by duplication of a hemoglobin gene). The outgroup in the analysis was the genus *Ateles*, the New World spider monkeys. The DNA data strongly indicate that chimpanzees and humans share a more recent ancestor with each other than they do with gorillas. (Figure 23.7), a conclusion supported by other types of molecular data.

Phylogenetic Trees Have Many Uses

Phylogenetic trees contain information that is useful to scientists investigating a wide variety of biological questions. Here we illustrate how phylogenetic trees are being used to determine how many times a particular trait may have arisen during evolution, and to assess when lineages may have split.

Proteins are the structures of proteins and nucleic acids (DNA and RNA).

protein structure. Relatively precise information about phylogenies can be obtained by comparison of the molecular structure of proteins. We can estimate genetic differences between two lineages by obtaining homologous proteins from both and determining the number of amino acids that have changed since the lineages diverged from a common ancestor.

DNA base sequences. The base sequences of DNA provide excellent evidence of evolutionary relationships among organisms. The cells of eukaryotes have genes in their mitochondria as well as in the nucleus; plant cells also have genes in their chloroplasts. The chloroplast genome (cpDNA), which is used extensively in phylogenetic studies of plants, has changed more slowly over evolutionary time. Mitochondrial DNA (mtDNA), which evolved much more rapidly than cpDNA, has been used extensively for evolutionary studies of animals. Relationships among apes and humans were investigated by sequencing more than 10,000 base pairs making up a segment of nuclear DNA that includes a he-

Numbers indicate the number of base-pair changes in the globin region of DNA.

Common ancestor

622 128

Spider monkey (*Ateles*)

128, i

Rhesus monkey

150 r—|

70

Orangutan

70 | 1

Y

14

Gorilla



] 76

Chimpanzee

Human

23.7 A Phylogeny of Anthropoid Primates

Analysis of DNA base pairs in the globin region indicates that humans and chimpanzees are more closely related to each other than they are to gorillas.

432 CHAPTER TWENTY-THREE

How Often Have Traits Evolved?

Most flowering plants reproduce by mating with another individual, outcrossing and have mechanisms to prevent self-fertilization. Many species, however, can fertilize themselves with their own pollen—they are self-compatible. How can we tell how often self-compatibility has evolved in a lineage? We can do so by plotting on a phylogenetic tree which species are outcrossing and which are selfing.

The evolution of fertilization methods was examined in *Linanthus* (a genus in the phlox family), a lineage of plants with a diversity of breeding systems and pollination mechanisms. The outcrossing (self-incompatible) species of *Linanthus* have flowers with long tubes and are pollinated by long-tongued flies. The self-fertilizing (self-compatible) species all have short-tubed flowers.

Investigators reconstructed a phylogeny for 12 species in a section of the genus using the internal-transcriber-spacer (ITS) region of nuclear ribosomal DNA (Figure 23.8). This region was known to be useful for reconstructing species-level phylogenies in other plant groups and had already been used for constructing a phylogeny of the phlox family. The investigators determined whether each species was self-compatible by artificially pollinating flowers with their own or outcrossed pollen and observing the results.

Several lines of evidence suggested that self-incompatibility is the ancestral state in limnites. First, multiple origins of self-incompatibility are not known in any other flowering plant family. Second, self-incompatibility systems involve physiological mechanisms in both the pollen and the stigma and require the presence of at least three distinct alleles. Therefore, a change from self-incompatibility to self-

CE2: I!pari' 1 ') 'y' c PaciprJj a an thn rrn^rcpV[-j pjngc. I hirrl in ? M

self-incompatible species of *Linanthus*, the site of pollen rejection is the stigma, even though sites of pollen rejection vary greatly among other plant groups.

Assuming that self-incompatibility is the ancestral state, the phylogeny suggests that self-compatibility has evolved three times in this *Linanthus* lineage (Figure 23.8). The change to self-compatibility has been accompanied by the evolution of reduced flower size. Interestingly, the striking similarity in flower form among the self-compatible groups had led to their classification as members of a single species. The phylogenetic analysis showed them to be members of three distinct lineages!

When Did Lineages Split?

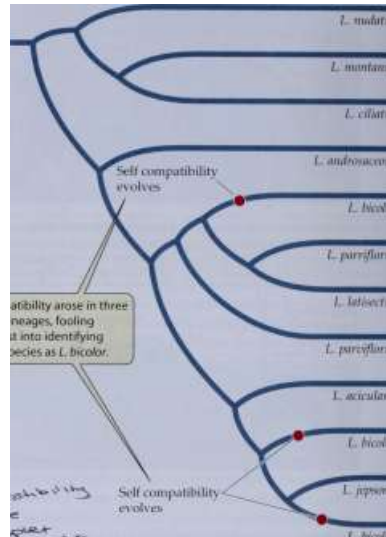
How fossils can help us determine evolutionary pathways is illustrated by studies of lungfishes. A phylogenetic tree of the

three extant genera of lungfishes, all of which are strictly limited to fresh water (Figure 23.9), indicates that the African *Protopterus* and the South American *Lepidosiren* (both in the family *Lepidosirenidae*) share a more recent common ancestor with each other than with the Australian *Neoceratodus* (family *Ceratodontidae*). Fossils of each genus are known only from the continent it now inhabits. By itself, this information suggests that the three genera were isolated by the breakup of Gondwana (see Chapter 20).

Common phlox

<£-

^jerr*?



Self-compatibility arose in three separate lineages, fooling taxonomists into identifying all three species as *L. bicolor*.

Self compatibility evolves

bicolor



8 Phylogeny of a Section of the Phlox Genus *Linanthus*

Self-compatibility apparently evolved three times in this lineage. Because the form of flowers converged in the selfing lineages, taxonomists mistakenly thought that they were all members of a single species.

However, fossils of other members of the family *Ceratodontidae* have been found in all continents except South America and Antarctica, and fossil *lepidosirenids* have been found in both Europe and North America. Thus, the ancestors of both families probably ranged over much of Pangaea. The combination of the phylogenetic tree and fossil evidence informs us that their divergence happened long before the breakup of Gondwana.

Why Classify Organisms?

Classification systems are important for several reasons. They improve our ability to explain relationships among things. They are also a mnemonic aid. It is impossible to remember the characteristics of many different things unless we can group them into categories based on shared characteristics. They are also useful as predictors. For example, the discovery of biochemical precursors of the drug cortisone in certain species of yams (genus *Dioscorea*) stimulated a successful search for higher concentrations of the drug in other *Dioscorea* species. And, as we saw at the be-



Common ancestor

23.9 Evolutionary Pathways in Lungfish Species

In this phylogeny, the ancestor is at the bottom of the figure.

Beginning of this chapter, a phylogeny of *Oncomelania* snails is helping to devise methods to control schistosomiasis.

Biological classification systems provide unique names for organisms. If the names are changed, the systems provide a means of tracing the changes. Common names, even if they exist (most organisms have none), are very unreliable and often confusing. For example, plants called bluebells are found in England, Scotland, Texas, and the Rocky Mountains—but none of the bluebells in any of those places is closely related evolutionarily to the bluebells in any of the other places (Figure 23.10).

Recognizing and interpreting similarities and differences among organisms is easier if the organisms are classified into groups that are ordered and ranked. Any group of organisms that is treated as a unit in a biological classification system is called a taxon (plural taxa). Taxonomy is the theory and practice of classifying organisms.

The Hierarchical Classification of Species

The biological classification system that is used today was developed by the Swedish biologist Carolus Linnaeus in 1758. His two-name system, referred to as binomial nomenclature, replaced the cumbersome descriptions biologists had previously used. For example, the honeybee, which had been named *Apis pubescens, thorace subgriseo, abdomine fuscato, pedibus posticis glabris utrinque margine ciliatis*, became simply *Apis mellifera*. Binomial nomenclature is universally employed in biology today. Using this system, scientists throughout the world refer to the same organisms by the same names.

Linnaeus gave each species two names, one identifying the species itself and the other the genus to which it belongs. A genus (plural genera; adjectival form, generic) is a group of closely related species. In many cases the name of the taxonomist who first proposed the species name is added at the end. Thus, *Homo sapiens* Linnaeus is the name of the modern human species. *Homo* is the genus to which the species belongs, and *sapiens* identifies the species; Linnaeus proposed the species name *sapiens*. You can think of the generic name *Homo* as equivalent to your surname and the specific name *sapiens* as equivalent to your first name. The generic name is always capitalized; the species name is not. Both names are always italicized, whereas common names are not.

w

(a) *Campanula* sp.



(c) *Endymion nonscriptus*

(c) *Mcrtensia virginica*

23.10 Many Different Plants Are Called Bluebells

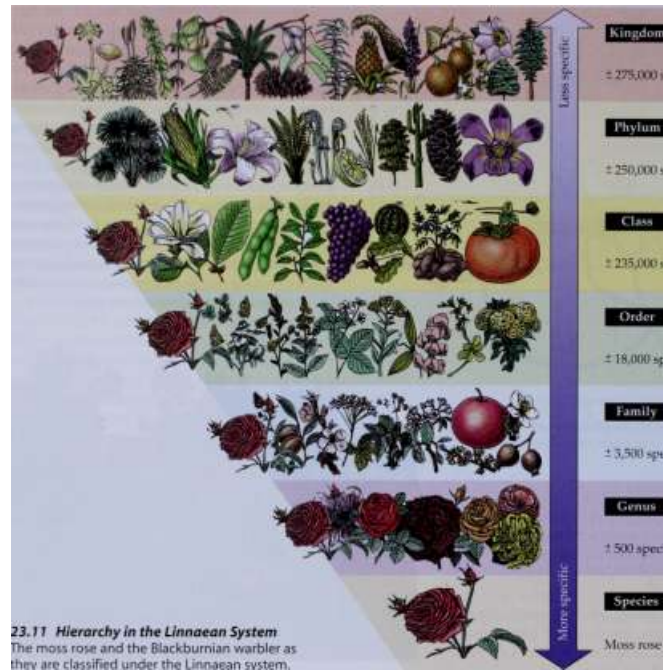
(a) These flowers from the plains of North Dakota are often called bluebells, (b) This English bluebell is a member of the lily family, (c) These are known as Virginia bluebells. None of these plants is closely related to the others.

434 CHAPTER TWENTY-THREE

± 275,000 species

± 250,000 species

± 235,000 species



± 18,000 species

± 3,500 species

± 500 species

Plantae

(plants)

Angiospermae (flowering plants)



Eudicotyledonae (true dicots)

Rosales (roses and their allies)

Rosaceae

Rosa

Rosa gallica

23.1 7 Hierarchy in the Linnaean System

The moss rose and the Blackburnian warbler as they are classified under the Linnaean system.

Moss rose

When referring to more than one species in a genus without naming each one, we use the abbreviation "spp." after the generic name (for example, "*Drosophila* spp." means more than one species in the genus *Drosophila*). The abbreviation "sp."

is used after a generic name if the identity of the species is uncertain. Rather than repeating a generic name when it is used several times in the same discussion, biologists often spell it out only once and abbreviate it to the initial letter thereafter (for example, *E. coli* is the abbreviated form of *Escherichia coli*).

In the Linnaean system, species and genera are grouped into higher taxonomic categories. The category (taxon) above genus in the Linnaean system is family. The names of animal families end in the suffix "-idae." Thus Formicidae-

ant species, and the fam-

dae is the family that contains all Hominidae contains humans, a few of our fossil relatives, and chimpanzees and gorillas. Family names are based on the name of a member genus. Formicidae is based on Formica, and Hominidae is based on Homo. Plant classification follows the same procedures except that the suffix

"-aceae" is used with family names instead of "-idae." Thus Rosaceae is the family that includes the genus of roses (*Rosa*) and its close relatives.

Families, in turn, are grouped into orders, and orders into classes. Classes are grouped into phyla (singular phylum), and phyla into kingdoms. The hierarchical units of this classification system, as applied to an animal species, the Blackburnian warbler (*Dendroica fusca*), and a plant species, the moss rose (*Rosa gallica*), are shown in Figure 23.11.

It should be obvious from this discussion that although the species category has real meaning and can be defined fairly rigorously, higher taxonomic categories are only mental constructs. They help us understand the diversity of life and its evolution, but they have only relative meaning. A family is always more inclusive than an order, and an order is more inclusive than a genus. However, there are no rigorous criteria by means of which to decide whether a particular lineage should be given the status of a family or an order. Therefore, an avian family may have a more recent common ancestor than a family of flowering plants, or vice versa.

RECONSTRUCTING AND USING PHYLOGENIES 435

Over 1,000,000 species

Animalia (animals)

Chordata (chordates)

+ 40,000 species

8,600 species

5,160 species

125 species

Aves (birds)

Passeriformes (songbirds)

Parulidae (wood warblers)

Dendroica

28 species

Dendroica fusca

Blackburnian warbler



Biological Classification and Evolutionary Relationships

Biological classification systems are designed to express relationships among organisms. The kind of relationship we wish to express influences which features we use to classify organisms. If, for instance, we were interested in a system that would help us decide what plants and animals were desirable as food, we might devise a classification system based on tastiness, ease of capture, and the type of edible parts each organism possessed. Early Hindu classifications of plants were designed according to these criteria. Biologists do not use such systems today, but they served the needs of the people who developed them.

Classification systems should be judged only in terms of their utility and consistency with their stated goals. To evaluate any classification system, we must first ask, What relationships is it trying to express? Then, How well does it express those relationships?

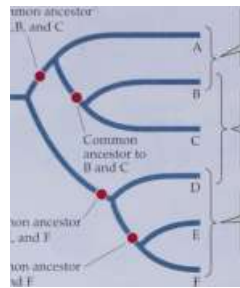
Early classifications were nonevolutionary

Many organisms were given species names and classified by Linnaeus and his followers before evolution became widely accepted as the central concept of biology. These naturalists described many features of organisms and grouped them according to the similarities that seemed most important. They tried to develop "natural" systems of classification, but they had no basis for deciding what was "natural" or why some features of organisms were more important than others.

Current biological classifications reflect evolutionary relationships

Most taxonomists today believe that classification systems should reflect the evolutionary relationships of organisms—that is, that taxonomic groups should be monophyletic. A monophyletic group (also called a clade) contains all the descendants of a particular ancestor and no other organisms. In other words, a monophyletic group is one that can be removed from a phylogenetic tree by one "cut" in the tree. A taxon consisting of members that do not share the same common ancestor is polyphyletic. A group that contains some but not all of the descendants of a particular ancestor is said to be paraphyletic (Figure 23.12).

Taxonomists agree that polyphyletic groups are inappropriate as taxonomic units. The classifications used today still contain many polyphyletic groups because many organisms have not been studied enough to distinguish between homologies and homoplasies. However, as soon as they detect homoplasies, systematists change their classifications to eliminate polyphyletic taxa. Thus the three lineages of self-compatible *Linanthus* shown in Figure 23.8 are now treated as distinct species.



A paraphyletic taxon includes some but not all descendants of a single ancestor.

A polyphyletic taxon contains members with more than one recent common ancestor.

Common ancestor to D, E, and F

Common ancestor to E and F

A monophyletic taxon includes all descendants of a single ancestor.



[171^ 23.12 Monophyletic, Polyphyletic, and Paraphyletic Taxa

Taxa are classified in terms of their evolutionary relationships. Polyphyletic groups are considered inappropriate as taxonomic units, but systematists sometimes use paraphyletic taxa.

In phylogenetic classification systems, formal taxonomic names are given only to monophyletic groups. But this does not mean that every monophyletic group should have a name. For example, it would be very cumbersome to put every pair of species into its own genus and every pair of genera into its own family. In addition, such a classification system would need to be changed every time a new species was described. Therefore, many monophyletic groups have no formal names. Systematists generally name only groups linked by many shared derived traits or by the presence of a major, obvious character that can be used to identify members of the group. These informal practices give stability to the classification system and aid in identifying organisms and their traits.

Although most systematists favor phylogenetic classifications, some believe that classification systems should also reflect degrees of difference "among organisms, not only thpjirj^vn hitinnary ppHig rpp ArrnrHing tn thm vipw mmps

should be retained for paraphyletic groups that have undergone rapid evolutionary change and diversification. The perspective of these taxonomists can be illustrated by birds, crocodilians, and their relatives.

We now know, from both fossil and anatomical evidence, that birds, turtles, and crocodilians (a group that includes crocodiles and alligators) share a more recent common ancestor than crocodilians and turtles share with snakes and lizards (Figure 23.13^). Traditionally, crocodilians were grouped with snakes, lizards, and turtles in the class Rep-tilia. Birds were placed in a separate class, Aves (Figure 23.13b). This classification came about because, since the time the two lineages separated, crocodilians have evolved more slowly than birds. As a result, crocodilians are more similar in many features to snakes and lizards than they are to birds. They look like very large lizards.

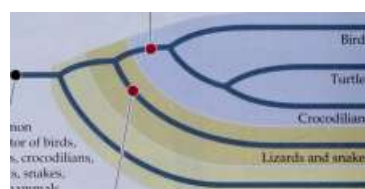
Figure 23.13b shows that the traditional class Reptilia is paraphyletic because it does not include all the descendants of its common ancestor; that is, birds are not included. If only monophyletic taxa were permitted, birds would be included with crocodilians, turtles, and their ancestors in a single taxon separate from snakes and lizards (Figure 23.13c). Retaining birds as a separate class (that is, retaining reptiles as a paraphyletic group) emphasizes that birds have undergone rapid evolution since they separated from reptiles and have developed major, unique derived traits.

The current tendency is to change classifications to eliminate paraphyletic groups, but-some of the most familiar taxonomic categories—gymnosperms and reptiles, for example—are paraphyletic. Because of their familiarity and the extensive literature devoted to them, these categories are likely to remain in use for some time, even after their formal taxonomic designations change.

(«) The evolutionary relationships

Common ancestor of

birds, turtles, and crocodilians



Common ancestor of birds, turtles, crocodilians, lizards, snakes, and mammals

Common

ancestor

of lizards

and snakes

(b) The traditional classification

Reptilia (reptiles)

Mammals

Aves (birds)

Crocodylians

Lizards and snakes

Mammalia (mammals)

Turtles

(c) A phylogenetic classification

Birds

Turtles

Crocodylians

Lizards and snakes

Mammals

23.13 Phylogeny and Classification

A phylogenetic classification based on their evolutionary relationships would group crocodylians and turtles together with birds. The traditional classification unites crocodylians and turtles with lizards and snakes in the paraphyletic taxon Reptilia because these animals share many morphological traits.

RECONSTRUCTING AND USING PHYLOGENIES 437

The Future of Systematics

The development of molecular methods and powerful computers has ushered in a new era of taxonomy. Computers enable systematists to analyze many characters and to compare many possible phylogenetic trees. Many phylogenies are being reconstructed, and classifications are being revised. Information from many sources continues to be used in constructing phylogenies. The range of data used in classification is likely to increase rather than decrease in the future because modern chemical, biochemical, and microscopic methods allow systematists to measure more traits of organisms than they could previously.

Often phylogenies are reconstructed as part of efforts to determine evolutionary relationships among organisms. In addition, as we have just seen, phylogenies are increasingly being used to answer many other types of biological questions. Many biological statements are phylogenetic statements. Any statement claiming an association between a trait and a group of organisms is a claim about when during a lineage the trait first arose and about the fate of the trait since its first appearance. For example, the statement that possession of a cytoskeleton is a trait possessed by all eukaryotes is a statement that the cytoskeleton is an ancestral, homologous trait that has been maintained during the subsequent evolution of all surviving eukaryote lineages.

Phylogenetic Trees Have Many Uses

► Phylogenetic trees help biologists determine how many times evolutionary traits have arisen and when lineages diverged. Review Figures 23.8, 23.9

Why Classify Organisms?

► Classification systems improve our ability to explain relationships among things, aid our memory, and provide unique, universally used names for organisms.

The Hierarchical Classification of Species

► Biological nomenclature assigns to each organism a unique combination of a generic and a specific name.

► In the Linnaean classification system, species are grouped into higher-level units called genera, families, orders, classes,

phyla, and kingdoms. Review Figure 23.11

Biological Classification and Evolutionary Relationships

► Taxonomists agree that taxa should share a common ancestor and that polyphyletic groups should not be used. Review Figure 23.12

► Paraphyletic taxa may be retained because of their familiarity and to highlight the fact that members of some lineages evolved especially rapidly. Review Figure 23.13

The Future of Systematics

► Molecular methods and powerful computers have ushered in a new era of systematics.

Chapter Summary

How Are Phylogenetic Trees Reconstructed?

► Phylogenetic trees display the patterns of evolution of life on Earth. In addition, they help biologists deal with a wide variety of practical problems. Review Figure 23.1

► Traits that are inherited from a common ancestor are said to be homologous. A derived trait is one that differs from its form in the ancestor of a lineage. Review Figure 23.2

► Traits that are similar as a result of convergent or parallel evolution or evolutionary reversals are said to be homoplastic. Review Figure 23.3

► To determine true evolutionary relationships, systematists must distinguish between ancestral and derived traits within a lineage, as well as between homologous and homoplastic traits. This task is often difficult because divergent evolution may make homologous traits appear dissimilar and convergent evolution may make homoplastic traits appear similar.

► Systematists often employ the principle of parsimony to reconstruct phylogenetic trees. Review Figure 23.5

Traits Used in Reconstructing Phylogenies

► Systematists use data from fossils and the rich array of morphological and molecular data available from living organisms to determine evolutionary relationships.

► Structures in early developmental stages sometimes show evolutionary relationships that are not evident in adults. Review Figure 23.6

► The structures of proteins and the base sequences of nucleic acids are important taxonomic data. Review Figure 23.7

For Discussion

1. The great blue heron, *Ardea herodias*, is found over most of North America. The very similar gray heron, *Ardea cinerea*, ranges over most of Europe and Asia. These two herons currently are treated as different species, but a colleague argues that they should be treated as a single species. What facts should you consider in evaluating your colleague's suggestion? What taxonomic theories would be relevant to your evaluation?

2. Why are systematists so concerned with identifying lineages that share a single common ancestor?

3. How are fossils used to identify ancestral and derived forms of traits of organisms?

4. Taxonomists regularly use the "parsimony principle" when reconstructing phylogenetic trees. Given that nature is not always parsimonious, why is parsimony used as a guiding principle?

5. A student of the evolution of frogs has proposed a strikingly new classification of frogs based on an analysis of a few mitochondrial genes from about 25 percent of frog species. Should frog taxonomists immediately accept the new classification? Why or why not?

6. Linnaeus developed his system of classification before Darwin proposed his theory of evolution by natural selection, and most early classifications of organisms were developed by non-evolutionists. Yet many of these classifications are still used today, with minor modifications, by most evolutionary taxonomists. Why?

24

Molecular and Genomic Evolution

► A group of extinct human relatives that lived in Europe and eastern Asia from about 300,000 to 30,000 years ago. During part of that time they coexisted with *Homo sapiens*. Some researchers have identified Neanderthals as direct ancestors of modern humans. Others believe that Neanderthals contributed only a few

genes to the human gene pool. Still others think that they contributed no genes.

In an attempt to discover which of these three hypotheses is correct, scientists extracted mitochondrial DNA from a section of a leg bone of a Neanderthal fossil between 30,000 and 100,000 years old. The base sequences of the Neanderthal mtDNA fell well outside the variation found in modern human mtDNA. From these results, investigators judged that Neanderthal mtDNA and modern human mtDNA have been evolving separately for at least 500,000 years. This finding provided evidence that Neanderthals contributed few or no genes to the human gene pool.

How can investigators compare the mtDNA of humans and Neanderthals? In this chapter we review how molecular biologists determine the structures of nucleic acids and proteins and use those structures to infer both the patterns and the causes of molecular evolution. With these insights, we explore how the functions of molecules change, where new genes come from, and the evolution of the genomes of organisms. Finally, we show how knowledge of the patterns of molecular evolution helps us solve other biological problems, including inferring phylogenetic relationships among organisms and determining how humans spread over Earth.

What Is Molecular Evolution?

The molecules of interest to molecular evolutionists are nucleotides, nucleic acids, amino acids, and proteins. Nucleic acids evolve by means of nucleotide base substitutions, which in turn result in changes in the amino acids they encode. Alterations in the structure and functioning of proteins result from changes in the ordering of the amino acids of which they are composed. Molecular evolutionists investigate the evolution of these macromolecules to determine how rapidly they have changed and why they have

Neanderthal Bones

DNA recovered from bones of Neanderthals can be used to infer whether Neanderthals contributed many or no genes to the modern human genome. This skeleton was unearthed in 1908 from a cave in France.

changed. To do so, they must be able to characterize the precise structures of these macromolecules.

Molecular evolutionists also try to reconstruct the evolutionary histories of genes and organisms, a field known as molecular phylogenetics. These two components of the study of molecular evolution are intimately related because phylogenetic information is essential for determining the order of changes in molecular characters, and knowing the order of such changes is usually the first step in inferring their causes. Conversely, knowledge of the pattern and rate of change in a given molecule is crucial for attempts to reconstruct the evolutionary history of a group of organisms.

For most of its history, evolutionary biology depended on the study of the obvious morphological features of organisms. During his 5-year voyage aboard the *Beagle*, Charles Darwin observed morphological differences among species found in different geographic areas. He later synthesized these observations into descriptions of how species change over time. He was able to hypothesize why



MOLECULAR AND GENOMIC EVOLUTION 439

many of these morphological changes had happened, but he could not determine how they occurred. Understanding the mechanisms of morphological change had to await discoveries in biochemistry a century later.

Even though genetic differences underlie all components of the adaptive evolution of organisms, molecular evolution differs from phenotypic evolution in one important way. In addition to natural selection, random genetic drift and mutation exert important influences on the rates and directions of molecular evolution.

A mutation, as you know from Chapter 12, is a change in the sequence of a single copy of a gene (see pages 234-235). A substitution is the partial or complete replacement of a nucleotide base or longer sequence by another throughout an entire population or species. It is substitutions that are of interest to molecular evolutionists.

Many mutations in sequences of genes do not alter the proteins encoded by those genes. The reason is that most amino acids are specified by more than one codon. Leucine, for example, is specified by six different codons: UUA, UUG, CUU, CUC, CUA, and CUG (see Figure 12.5; in this and all other cases, most of the redundancy is in the third codon position).

When it occurs throughout a population, a nucleotide substitution that does not change the amino acid specified—UUA to UUG, for example—is known as a synonymous or silent substitution. Synonymous substitutions are unlikely to affect the functioning of the protein (and hence the organism) and are therefore unlikely to be influenced by natural selection.

Because they are unlikely to be influenced by natural selection, synonymous substitutions are free to accumulate in a population over evolutionary time at rates determined by rates of mutation and genetic drift. Because modern molecular techniques enable us to detect substitutions at the level of nucleotides, molecular evolutionists can measure even these nonfunctional changes.

The occurrence in a population of nucleotide substitutions that do change the amino acid that is specified—UUA to UCA, for example, which would result in serine rather than leucine—is known as nonsynonymous substitution. In general, nonsynonymous mutations are likely to be deleterious to the individual organism. But even an amino acid change does not necessarily change a protein's shape and, hence, its functional properties. Therefore, a nonsynonymous substitution may be selectively neutral, or nearly so.

Most natural populations of organisms harbor much more genetic variation than we would expect if genetic variation were influenced primarily by natural selection. This discovery, combined with the knowledge that many substitutions do not change molecular function, stimulated the development of the neutral theory of molecular evolution.

The neutral theory, first articulated by Motoo Kimura in 1968, postulates that, at the molecular level, the majority of mutations are selectively neutral: they confer neither an advantage nor a disadvantage on their bearers. If so, the majority of evolutionary changes in macromolecules, and much of the genetic variation within species, results from

neither positive selection of advantageous alleles nor stabilizing selection, but from random genetic drift.

To see why this is so, consider a population with a size of N and a rate of neutral mutation at a particular locus of μ per gamete per generation. The number of new mutations would on average be $\mu \times 2N$, because $2N$ gene copies are available to mutate. According to genetic drift theory (see Chapter 21), the probability that a mutation will be fixed by genetic drift is its frequency, p , which equals $1/(2N)$ for a newly arisen (and hence very rare) mutation. Therefore, the number of neutral mutations that arise per generation that are likely to become fixed is $2N\mu \times 1/(2N) = \mu$, which equals the mutation rate.

In other words, the rate of fixation of mutations is theoretically constant and is equal to the neutral mutation rate. This is the theoretical basis of the concept of the molecular clock, which states that macromolecules should diverge from one another over time at a constant rate. We will discuss molecular clocks later in this chapter and show how, with care, the concept can be used to study many features of molecular evolution.

According to the neutral theory of molecular evolution, most polymorphisms at specific genetic loci are transitory rather than stable, because the frequency of neutral alleles in a population should change slowly over time (Figure 24.1a). In contrast, advantageous mutations are rapidly fixed in a population, and deleterious mutations are quickly lost (Figure 24.1fc). The neutral theory and the theory of natural selection agree that most mutations are deleterious, but the neutral theory asserts that the selective ad-

(«)

c

QJ

3

o)

The frequency of neutral alleles changes very slowly.



Time

(b)

c

3 01

/

Advantageous mutations are quickly fixed in the population...



...while deleterious mutations are quickly lost.

Time

24.1 Allele Frequencies Change at Different Rates

(a) The frequencies of neutral alleles change slowly. Much polymorphism in these alleles is transitory, (b) Alleles carrying advantageous mutations become fixed in populations while disadvantageous mutations are eliminated; these allele shifts usually occur rapidly.

440 CHAPTER TWENTY-FOUR

Advantages or disadvantages of most molecular mutations are so small that selection on them is too weak to offset the influences of genetic drift.

Determining and Comparing the Structure of Macromolecules

To reveal patterns of molecular evolution, biologists may determine the precise structure of biological molecules. An investigator attempting to determine the structure of a nucleic acid or a protein begins by extracting and purifying it from a natural source. The molecule can then be analyzed by X ray crystallography. The molecule is crystallized, and the crystal is bombarded with a beam of X rays. The regularly spaced atoms in the crystal deflect the X rays into an orderly array of spots on a photographic film. With data from successive cross-sections through the crystal, a computer can generate a three-dimensional electron density map of the molecule. Graphics software enables the computer to create a picture showing the position of each atom in the molecule (Figure 24.2).

The base sequences of nucleic acids also provide important information about evolutionary histories. The invention of the polymerase chain reaction (PCR) technique (see Chapter 11) allowed biologists to determine the sequence of regions of DNA not only from living tissues, but also from fossilized remains, mummified tissues, dried skins in museums, and pressed plants in herbaria, even though these objects contain only tiny amounts of DNA. DNA has been extracted and amplified from human fossils more than

RESEARCH METHOD



24.2 Computer Graphic Shows the Positions of Atoms in Molecules

The positions of atoms and the three-dimensional structure of tuna cytochrome c were computed from data generated by cross-sections through the crystallized molecule.

Q Two amino acid sequences seem quite different...

Sequence 1 • • • • dgfMffWgMg-MgjM gfr ^jg* • Sequence 2 • • • -<||fHfflj<j%iijg.-Htfift^fr. • • •

...but if we insert a gap in sequence 2, there is nearly complete alignment.

Sequence 1 Sequence 2

t

With this alignment established, we can compare additional sequences.

Sequence 1 Sequence 2 Sequence 3 Sequence 4 Sequence 5 Sequence 6

®®®®ooo

tA similarity matrix provides a count of similar and differing amino acids for each pair of sequences.

f

Numbers above the diagonal line are differences.

Sequence number 13 4 5

5 2

\$3

c

% 4

f

Numbers below the diagonal line are similarities.



t 24.3 Amino Acid Sequence Alignment

Inserting a gap allows us to align two sequences so that we can compare homologous amino acids. Once the alignment is established, more sequences can be added and compared. The larger the number of similarities, the more recent the presumed common ancestor of the species.

30,000 years old, plant leaf fossils 40,000 years old, and insects fossilized in amber 135,000,000 years ago.

Once the sequences of amino acids in molecules from different organisms have been determined, they must be compared. A simple example illustrates how this is done. In Figure 24.3, two amino acid sequences (1 and 2) are

compared. The two sequences come from homologous proteins in different organisms, and they differ in number and identity of amino acid residues. Our goal is to align these sequences so that we can compare homologous portions of the protein. To do so, we first observe that, although the sequences appear quite different, they would become similar if we were to insert a gap after the first amino acid in sequence 2 (after the leucine residue). In fact, these sequences then differ by only one amino acid at position 6 (serine or phenylalanine). A single insertion aligns the sequences in this case, but longer sequences and those that have diverged more extensively require more elaborate adjustments.

After we have aligned the sequences, we can compare them in several ways. First, we can simply count the number of nucleotides or amino acids that differ between the sequences. Let's add some more sequences to our previous example and compare them with our original two sequences. By adding up the number of similar and different amino acids in the sequences, we can construct a similarity matrix (see Figure 24.3). The assumption is that the longer the molecules have been evolving separately, the more differences they will have.

Enough analyses of mammalian genes have been performed to show that the rate of nonsynonymous nucleotide substitution in mammals varies from nearly zero to about 3×10^{-9} substitutions per site per year. Synonymous substitutions in the protein-coding regions of nuclear genes have occurred about 5 times more rapidly than nonsynonymous substitutions; in other words, substitution rates are highest at codon sites that do not change the amino acid being expressed (Figure 24.4). The rate of substitution is even higher in pseudogenes—duplicate copies of genes that have undergone one or more mutations that eliminate their ability to be expressed.

Rates of substitution are high where they do not affect functioning...

Pseudogenes

Synonymous substitutions

Nonsynonymous substitutions



...and are low where they change the amino acid being expressed.

12 3 4

Substitutions per nucleotide site per 10 million years

24.4 Rates of Base Substitution Differ

Rates of nonsynonymous substitutions in mammals are much slower than rates of synonymous substitutions and substitutions in pseudogenes.

Why do rates of nucleotide substitution vary so greatly?

The fact that rates of nucleotide substitution are highest at sites and in molecules where they have no functional significance is consistent with the hypothesis that substitution rates at these sites are driven primarily by a combination of mutation and genetic drift. The much slower rates of substitution at sites that do affect molecular function is consistent with the view that most nonsynonymous mutations are disadvantageous and are eliminated from the population by natural selection. An interesting consequence of these processes is that, in general, the more essential a molecule is for cell functioning, the slower the rate of its evolution.

A molecule that illustrates this principle is the enzyme cytochrome c, one component of the respiratory chain of mitochondria. Together with other proteins of the citric acid cycle and respiratory chain, cytochrome c is found in all eu-karyotes. The amino acid sequences of cytochrome c are known for more than 100 species of organisms, including microbial eukaryotes, plants, fungi, and mammals. Within these cytochromes c are regions that accumulated changes relatively quickly; for example, positions 44, 89, and 100 differ among many of the organisms compared (Figure 24.5 on pages 442-443).

There are also invariant positions, such as 14,17,18, and 80. This particular set of invariant residues is known to interact with the iron-containing heme group that is essential for the functioning of the enzyme. Presumably, because any mutations that changed these amino acids diminished the functioning of the heme group, they were removed by natural selection when they arose.

Using biological molecules as molecular clocks

Earlier in this chapter, we stated the theoretical basis for expecting macromolecules to evolve at constant rates. But do they actually behave as the theory says they should? For example, if we plot the time since the divergence of certain organisms, as determined by the fossil record, against the number of amino acids by which their cytochromes c differ, we find that differences in cytochrome c sequences have evolved at a relatively constant rate (Figure 24.6).

Many other proteins show constancy in the rate at which they have accumulated changes over time. It would be convenient if the rates of change were the same for all protein molecules. Unfortunately, different molecular clocks tick at different rates. These differences exist because proteins differ in the nature of functional constraints on their evolution.

Despite these differences, the rates at which many molecular clocks tick appear to be relatively constant. This is especially true for nucleotide or amino acid substitutions that do not affect the functioning of the molecule and, hence, the fitness of the organism. Even if the rate of ticking of a molecular clock changes slightly over time, the variations may not be great enough to seriously affect our estimates of the dates of divergences of gene and organism

442 CHAPTER TWENTY-FOUR



The number 1 indicates an invariant position in the cytochrome c molecule (i.e., all the organisms have the same amino acid in this position) and that the position is probably functionally very significant.

Side chains marked by red arrows interact with the heme group.

Tuna



Rice

Acidic side chains:

El Aspartic acid [E] Glutamic acid

Basic side chains:

o Histidine E Lysine E Arginine Hydrophobic side chains:

\Y\ Phenylalanine Q] Isoleucine \h\ Leucine S3 Methionine

Position in sequence

Number of amino acids in different
organisms at the position shown

Human, chimpanzee

Rhesus monkey

Horse

Donkey

Cow, pig, sheep

Dog

Rabbit

Gray whale

Gray kangaroo

Chicken, turkey

Pigeon

Pekin duck

Snapping turtle

Rattlesnake

Bullfrog

Tuna

Dogfish

[V] Valine [y] Tyrosine [w] Tryptophan [a] Alanine

Other:

Cysteine

Proline

Glutamine

Asparagine

Serine

Threonine

Glycine

Samia cynthia (moth)

Tobacco hornworm moth

Screwworm fly

Drosophila (fruit fly)

Baker's yeast

Candida krusei (yeast)

Neurospora crassa (mold)

Wheat

Sunflower

Mung bean

Rice

Sesame



10

3 4 14 3

I u

15

20

25

3 1 12434234214

30

1 2

24.5 Amino Acid Sequences of Cytochrome c

The two computer graphics show how similar the three-dimensional structure of tuna and rice cytochrome c are. The amino acid sequences shown here were obtained from analyses of cytochromes c from 33 species of plants, fungi, and animals.

lineages. By comparing the rates of a variety of molecular clocks, further insights can be gained into why different protein molecules have evolved at such different rates.

Where Do New Genes Come From?

The earliest forms of life must have had very few organized nucleic acid sequences. Because we believe that life is monophyletic—that all living organisms arose from a single ancestor—the many thousands of different functional genes in modern organisms must have arisen from these few ancestral genes. How has this happened? By far the most important process appears to be gene duplication.

Gene duplication may involve part of a gene, a single gene, parts of a chromosome, an entire chromosome, or the whole genome (see Chapter 14). We saw in Chapter 22 that duplication of the entire genome (polyploidy) has been important in speciation. Polyploid individuals are usually vi-

able because all of their chromosomes are duplicated, so that they avoid imbalances in gene expression. As we have already discussed, polyploidy is widespread among plants. Genome duplication was probably widespread among animals before the sex chromosomes became differentiated. Among organisms with differentiated sex chromosomes, however, genome duplication disrupts the mechanisms of sex determination.

Duplications of part or all of a chromosome are probably unimportant as sources of new genes because they typically result in severe imbalances in gene products. *Drosophila* in which more than half of one arm of a chromosome is present in three doses (trisomy) do not survive. In humans, trisomies larger than one chromosome are lethal; even smaller ones result in sterility. For example, individuals having three copies of chromosome 21 have Down syndrome, and are usually sterile. Therefore, duplications of whole chromosomes or parts of chromosomes generally are not passed along to any offspring.

Duplication of genes can lead to new gene families

The two identical copies of a gene produced by gene duplication may retain their original function, with the result that the organism produces larger quantities of their RNA

Multiple amino acids at a position indicate a great deal of change and that the position is probably less significant.

35

40

45

50

55

60

65

70

MOLECULAR AND GENOMIC EVOLUTION 443

Mostly Rarely

Invariant Uncharged charged Uncharged hydrophobic

75

80

85

90

95

100 104

or protein product. Alternatively, one copy may be incapacitated by the accumulation of deleterious mutations and become a functionless pseudogene. More importantly for evolution, one copy may retain its original function while the other accumulates enough mutations that it can perform a different task. Several successive rounds of duplication may result in a gene family, a group of homologous genes with related functions. Members of a gene family are often arrayed in tandem along a chromosome.

Molecular evolution by gene duplication has been well studied in the globin gene family (see Chapter 14). Globins were among the first proteins to be sequenced and their amino acid sequences compared. Humans have three families of globin genes: the myoglobin family, whose single member is located on chromosome 22; the α -globin family, on chromosome 16; and the β -globin family, on chromosome 11 (see Figure 14.9).

Two types of proteins are produced by these three gene families: myoglobin and hemoglobin. Comparisons of their

24.6 Cytochrome c Molecules Evolved at a Constant Rate

Rates of substitution in cytochrome c are constant enough that this molecule can be used as a molecular clock.

E

o

o

70

60

8 50

40

0>

■ z

30

20

.5 10

i

<

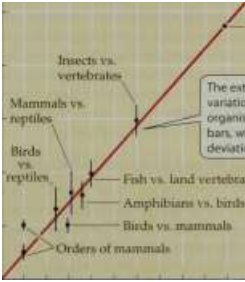
In the short term there is variability in the clock rate, but cytochrome c provides a remarkably constant molecular clock over long evolutionary time.

Angiosperms vs. animals



Insects vs. vertebrates

Mammals vs. reptiles



Birds

vs. reptiles

,•—Yeast vs. molds

The extent of intertaxon variation is shown for organisms by the vertical bars, which plot mean deviations from the average.

Fish vs. land vertebrates Amphibians vs. birds and mammals Birds vs. mammals

'Orders of mammals

200 400 600 800 1000 1200 1400 Time since divergence (millipns of years)

444 CHAPTER TWENTY-FOUR

Ancestor myoglobin-like molecule

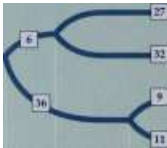


257" Myoglobin

81

120

Numbers indicate the estimated number of DNA sequence changes along the given branch of a tree.



Alpha chains (<x1, o2)

Zeta chain

(o

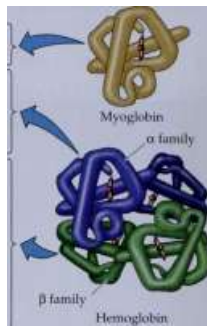
Epsilon chain (e)

Gamma chains

(*y, G y)

Delta chain (5)

Beta chain (P)



Hemoglobin

Precambrian Cambrian Ordovician Silurian Devonian Carboniferous Permian Triassic

Cretaceous

Tertiary

409 354 290 245 206

Millions of years ago (mya)

Present

24.7 A Globin Gene Tree

The globin family gene tree suggests that myoglobin diverged from modern hemoglobin precursors about 500 mya, at about the time of the origin of vertebrates.

amino acid sequences strongly suggest that rather than arising from different genes that independently converged on similar functions, the different forms of globins arose through gene duplications. How long the globins have been evolving separately can be inferred by comparing their amino acid sequences. The greater the number of amino acid differences between two globins, the farther back in time was their most recent common ancestor.

To estimate the time of the first globin gene duplication, we can create a gene tree, similar to a phylogenetic tree. Our tree is based on the estimated number of base substitutions necessary to account for the observed amino acid differences between the globins. Based on this tree, the earliest organisms known to have both myoglobin and hemoglobin must have lived about 500 million years ago. Thus, the initial duplication event by which myoglobins diverged from all other globins probably happened at least that long ago (Figure 24.7). Assuming that the rate of amino acid substitution has been relatively constant since then—about 100 substitutions per 500 million years—the α and β hemoglobins are estimated to have split about 450 mya.

Homologous genes may be found in distantly related organisms

How can we tell whether genes in different species are really homologous? One way to detect homologous genes in distantly related organisms is to find identical or nearly identical families of genes that produce similar effects in a wide variety of organisms. The homeotic gene complex is one such family.

As we saw in Chapter 16, some mutations in *Drosophila* cause appendages that are appropriate to one body segment to appear in another. Thus, leglike appendages may grow where there should be antennae, or a body segment may be duplicated (Figure 24.8). These unusual changes are caused by genes that occur in two tightly linked clusters that together constitute the homeotic gene complex.

All homeotic genes contain a region called the homeo-box, which in *Drosophila* specifies a sequence of 60 amino acids. Homeobox genes (Hox genes for short) are responsible for turning many other genes on or off. They are crucial in that they specify body segments and, in the absence of mutations, are responsible for the appropriate development of those segments.

This pattern of developmental control is not unique to fruit flies (see Figure 16.17). More than 350 homeobox elements have been identified in cnidarians, fungi, plants, and animals. Sponges, the simplest of multicellular animals, have only one homeobox-like gene; sea anemones (cnidarians) have up to seven. Vertebrate Hox gene clusters can have as many as thirteen genes. Hox genes occur in the same order along the chromosomes in all animals.

Hox genes carry out similar functions in all animals. A gene found in a simple cnidarian, the hydra, coordinates development of the animal's tentacles. This finding suggests that early in evolution, before animals had strongly developed head, body, and tail regions, Hox genes may have worked to specify the axis of development of the body. Structural and functional similarities strongly suggest that all homeobox genes have a common evolutionary origin,

Normal *Drosophila*

Second thoracic segment

Third thoracic segment



The third thoracic segment is mutated to produce an extra second thoracic segment.

bithorax mutation



24.8 The bithorax Mutation in Drosophila

Mutations of one of the homeotic genes, bithorax, transform the third thoracic segment into a second copy of the second thoracic segment. The result is a fly with two pairs of wings.

and that the mechanisms that determine the differentiation of the major morphological regions (body, head, trunk, and tail) may have arisen only once in animal evolution.

How Do Proteins Acquire New Functions?

Evolution as we know it would not have been possible if proteins were unable to change their functional roles. Gene duplication frees one copy of a gene from having to perform its original function. The copy is redundant because the original protein is still encoded by the original gene. Therefore, duplication allows the evolution of entirely novel functions.

Gene families provide evidence of functional diversification

For an example of how gene duplication permits the genes, and the proteins they encode, to evolve different functions, let's look again at the globin families. Hemoglobin, a tetramer consisting of two α chains and two β chains, carries oxygen in the blood. Myoglobin, a monomer, is the primary oxygen storage protein in muscle. It has evolved an affinity for oxygen that is much higher than that of hemoglobin.

In contrast to myoglobin, hemoglobin evolved to be much more refined and diversified in its role as the blood oxygen carrier. Hemoglobin binds oxygen from the lungs or gills, where the oxygen concentration is relatively high,

MOLECULAR AND GENOMIC EVOLUTION 445

transports it to regions of low oxygen concentration, and releases it in those areas. With its more complex, tetrameric structure (see Figure 3.7), hemoglobin also is able to transfer hydrogen ions and carbon dioxide in the blood and bind together four molecules of oxygen.

In humans, the α -globin family has four functional genes and three pseudogenes. The four functional genes diversified in function, while the three pseudogenes lost all function. Thus, duplication events may result in increased genomic complexity (as seen with the alternate genes for α -globin) as well as nonfunctional DNA (pseudogenes).

The globin genes show that molecular functions may change after gene duplication, but how do these functional changes happen? We will explore this interesting component of molecular evolution by using lysozyme as an example.

Lysozyme evolved a novel function

Lysozyme is an enzyme found in almost all animals. It is produced in the tears, saliva, and milk of mammals and in the whites of bird eggs. Lysozyme digests the cell walls of bacteria, rupturing and killing them. As a result, lysozyme plays an important role as a first line of defense against invading bacteria. All animals defend themselves against bacteria by digesting them, which is probably why all animals have lysozyme. Some animals, however, also use lysozyme in the digestion of food.

Among mammals, a novel mode of digestion called foregut fermentation has evolved twice. The anterior part of the stomach (the foregut) has been converted into a chamber in which bacteria break down ingested plant matter by fermentation. Mammals with this adaptation can obtain nutrients from the otherwise indigestible cellulose of plant material.

Foregut fermentation evolved independently in ruminants, such as cows, and certain leaf-eating monkeys, such as langurs. We know that these evolutionary events were independent because close relatives of langurs and ruminants do not ferment their food in the foregut. In both foregut-fermenting lineages, lysozyme has been modified to play a new, nondefensive role in the foregut. Lysozyme ruptures some of the bacteria that live in the foregut, releasing nutrients, which the mammal absorbs.

How many changes were incorporated into the lysozyme molecule to allow it to function amid the digestive enzymes and acidic conditions of the mammalian foregut? To answer this question, molecular evolutionists compared the amino acid sequences of lysozyme in foregut fermenters and in several of their nonfermenting relatives. They then determined which

amino acids differed and which were shared among the species (Table 24.1). Finally, they compared the patterns of these changes with the known phylogenetic relationships among the species.

The most striking finding is that amino acid changes have occurred about twice as rapidly in the lineage leading to langur lysozyme as in any other primate lineage. This high rate of substitution shows that lysozyme went

446 CHAPTER TWENTY-FOUR

Shown above the diagonal line is the number of amino acid sequence differences between the two species being compared; below the line are the number of sequences uniquely shared by the two species. Asterisks (*) indicate foregut-fermenting species.

through a period of rapid adaptation to the stomachs of langurs. The lysozymes of langurs and cows share five amino acid substitutions, all of which lie on the surface of the lysozyme molecule, well away from the active site. Several of the shared substitutions involve changes from arginine to lysine, which makes the lysozymes more resistant to attack by the pancreatic enzyme trypsin. By understanding the functional significance of amino acid substitutions, molecular evolutionists can explain observed changes in amino acid sequences in terms of the changing function of the protein.

A large body of fossil, morphological, and physiological evidence shows that langurs and cows do not share a recent common ancestor. However, langur and ruminant lysozymes share many amino acid residues that neither animal shares with the lysozymes of their own closer relatives. The lysozymes have converged on a similar sequence despite having very different ancestry; in other words, they are homoplasies. The amino acid residues they share give these lysozymes the ability to lyse the bacteria that ferment leaves in the foregut.

An even more remarkable story emerges if we look at lysozyme in the crop of the hoatzin, a leaf-eating South American cuckoo, the only known avian foregut fermenter. Hoatzins have an enlarged crop that contains resident bacteria and acts as a fermenting chamber. Many of the amino acid changes that occurred in the adaptation of hoatzin crop lysozyme are identical to the changes that evolved in ruminants and langurs. Thus, even though these three groups have evolved independently from one another for more than 300 million years, they have each evolved a similar molecule that enables them to recover nutrients from their fermenting bacteria in a highly acidic environment. The lysozyme story also illustrates why using single molecules to infer phylogenetic histories can be very misleading.

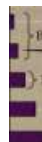
Genome Organization and Evolution

Investigations of the sizes and compositions of the genomes of many species have revealed tremendous variation. Multicellular organisms have more DNA than do single-celled organisms. The genome of *Mycoplasma genitalium*, the simplest free-living prokaryote, has only 470 genes. *Rickettsia prowazekii*, the prokaryote that causes typhus, has 634 genes. The genome of the yeast *Saccharomyces cerevisiae*—a eukaryote—has about 6,000 genes; that of the nematode *Caenorhabditis elegans* has about 20,000 genes (Figure 24.9).

It is not surprising that more complex instructions are needed for building and maintaining a large, complex organism than a small, simpler one. What is surprising is that lungfishes and lilies have about 40 times as much DNA as humans do. Clearly, a lungfish or a lily is not 40 times more complex than a human. Why does genome size vary so enormously among organisms? How did this variation arise? What fraction of genomes consists of coding DNA? Does the noncoding fraction have a function, or is it "junk?"

Some of the apparent differences in genome size disappear when we compare the portion of DNA that actually encodes functional RNA's or proteins. The size of the coding genome varies in a way that makes sense. Eukaryotes have more coding DNA than prokaryotes; vascular plants have more coding DNA than single-celled organisms; invertebrates with wings, legs, and eyes have more coding DNA than roundworms; and vertebrates have more DNA than invertebrates. The species with the largest amount of nuclear DNA has 80,000 times as much as the simplest organisms, but the species with the largest number of genes has only 20 times as many genes as a bacterium. Therefore, most of the variation in genome size is not due to differences in the number of functional genes, but in the amount of noncoding DNA (Figure 24.10).

What maintains such large quantities of noncoding DNA in the cells of most organisms? Most of this DNA appears to be nonfunctional. Much of it may consist of pseudogenes that are simply carried in the genome because



Bacteria

Dark bars show results based on complete I genome sequencing.

Fungi

* Invertebrates



Lighter bars are estimates based on sequencing samples.

H. influenzae

E. coli

Yeast

Drosophila

C. elegans (nematode)

Sea squirt

Pufferfish

Mouse

Human

0 25 50 75 100

Number of genes X 1,000

24.9 Complex Organisms Have More Genes than Simpler Organisms

Genome sizes have been measured or estimated in a variety of organisms, ranging from single-celled prokaryotes to vertebrates.

► Vertebrates



c

bo

c o

•J2

(o

100

80

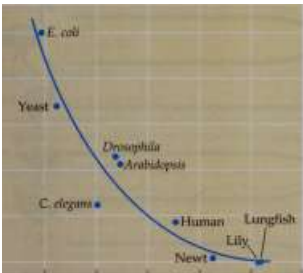
60

40

Z 20

o

Yeast



C. elegans m

0.001 0.01 0.1 1 10 100 1000

Genome size (x 10^9 base pairs)

24.10 Some DNA Does Not Code for Genes

Most of the DNA of bacteria and yeast codes for genes, but most of the DNA of more complex organisms is noncoding. We do not know how much of this noncoding DNA is nonfunctional.

the cost of doing so is small. Some of the DNA may be parasitic transposable elements (see Chapter 14) that spread through populations because they reproduce faster than the host's genome. Nonetheless, it is still possible that some of this DNA has undetected functions.

Using Biological Molecules to Reconstruct Phylogenetic Trees

By comparing the structures of molecules from different species, we both gain insights into how the molecules function and acquire a tool for inferring phylogenies. Molecules that have evolved slowly can be used to estimate relationships among organisms that diverged long ago. Molecules that have evolved rapidly are useful for studying organisms that share more recent common ancestors.

As we have seen, there is much evidence to suggest that sequences of amino acids in proteins or base sequences in RNA and DNA that have changed very little during evolution probably have the same function in all species. Sequences that have changed rapidly during evolution either have less important functions in the cell or have undergone major changes in function, as happened to lysozyme in foregut fermenters.

If you are interested in determining the evolutionary relationships of all existing organisms, you must choose a molecule that all organisms possess, such as ribosomal RNA. Equally important, rRNA experiences strong functional constraints; that is, even minor changes in the rRNA sequence prevent ribosomes from functioning properly. As a result, rRNA has evolved so slowly that comparisons of differences among the rRNA's of living organisms can be used to estimate lineage splits that may have happened billions of years ago.

Although molecular data are often the only data available with which to estimate the timing of ancient lineage divisions or reconstruct the phylogenies of prokaryotes,

MOLECULAR AND GENOMIC EVOLUTION 447

molecular data are also regularly used in combination with morphological and fossil data. Why do we use molecules when morphology is available? The answer is simple: The more characters that are used (morphological, molecular, fossil, and so on) to reconstruct a phylogeny, the less likely we are to be misled by losses of traits or convergent evolution, as we discussed in Chapter 23.

The more types of molecules we use, the better we can detect homoplasies. For example, if we were to infer a phylogeny only from lysozyme sequences, we might falsely conclude that langurs, cows, and hoatzins are all closely related. However, if we compared the structures of many molecules in those animals, even in the absence of morphological and fossil data, it is clear that these species do not share a recent common ancestor.

No fossils exist to document the most ancient splits in the lineages of life. Molecular evolutionists have used small-subunit rRNA molecules, which are found in all organisms and evolve very slowly, to infer the times of these lineage separations. These rRNA's strongly support the division of living organisms into three major branches, or domains: the Bacteria, the Archaea, and the Eukarya.

The structure of DNA extracted from extinct organisms is being used to determine the evolutionary relationships between those organisms and their surviving relatives. For example, DNA was obtained from the bones and mummified soft tissues of moas—large, flightless birds (weighing up to 200 kg) that lived in New Zealand until humans arrived a thousand years ago and hunted them to extinction. Comparison of their DNA with the DNA of other groups of flightless birds, such as kiwis and rheas, suggests that although kiwis and moas both lived in New Zealand, they are not each other's closest relatives (Figure 24.11). The closest relatives of the moas are unknown, extinct flightless birds that also gave rise to flightless descendants in Australia. Kiwis came to New Zealand more recently; their closest relatives, emus and cassowaries, live in their ancestral home, Australia and New Guinea.

Molecular Studies of Human Evolution

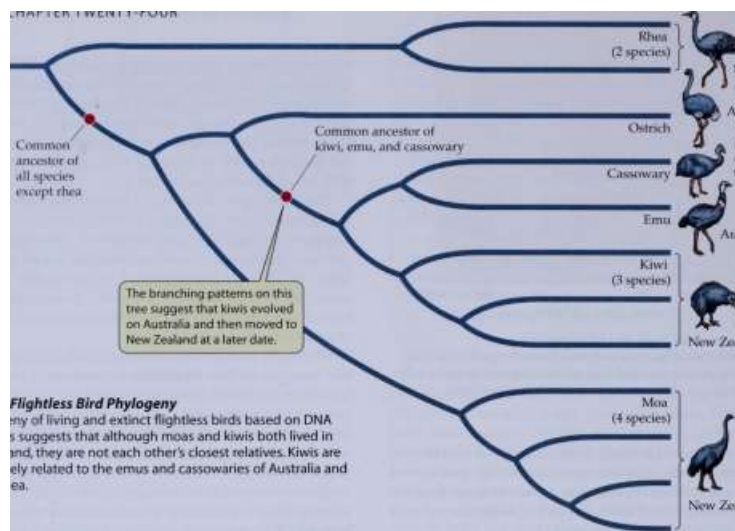
Molecular data have influenced our understanding of our own evolution. Fossil evidence suggests that the hominoid lineage leading to modern humans diverged from a chimpanzee-like lineage about 5 million years ago in Africa. About 2 mya, the human ancestor known as *Homo erectus* arose in Africa, then spread to other continents. Fossil remains of *Homo erectus* have been found in Africa, Indonesia, China, the Middle East, and Europe.

The transition from *Homo erectus* to *Homo sapiens* probably occurred about 400,000 years ago, but there is considerable controversy about the place of origin of modern humans. The "out of Africa" hypothesis suggests a single origin in Africa followed by several dispersals. The "multiple regions" hypothesis, in contrast, proposes several earlier origins of *Homo sapiens* from *Homo erectus* in different regions of Europe, Africa, and Asia (Figure 24.12).

448 CHAPTER TWENTY-FOUR

Common ancestor

Common ancestor of all species except rhea



South America

Africa

Australia and New Guinea

Australia

The branching patterns on this tree suggest that kiwis evolved on Australia and then moved to New Zealand at a later date.

24.11 A Flightless Bird Phylogeny

A phylogeny of living and extinct flightless birds based on DNA sequences suggests that although moas and kiwis both lived in New Zealand, they are not each other's closest relatives. Kiwis are more closely related to the emus and cassowaries of Australia and New Guinea.

The limited number of human fossils and their patchy distribution do not allow us to choose between these two hypotheses. However, DNA sequences of several mitochondrial genes from individuals from more than 100 ethnically distinct modern human populations have provided valuable evidence. Mitochondrial DNA (mtDNA) is useful for studying the recent evolution of closely related species and populations because it accumulates mutations rapidly and because it is maternally inherited. The Y chromosome serves the same role for following male lineages.

The mtDNA sequences of modern humans imply a common ancestry of all mtDNA's about 200,000 years ago. This date of shared ancestry was calculated using the number of nucleotide differences among existing humans; the rate of mtDNA sequence divergence was calibrated using mammals with better fossil records.

The multiple-origins hypothesis requires at least 1 million years of divergence since the last common ancestor. Thus, the mtDNA analysis lends support to the "out of Africa" hypothesis, suggesting that all modern human populations share a recent mitochondrial ancestor. Studies of 26 nuclear genes and a family of repeated sequences (called the Alu family), as well as analysis of sequences on the Y chromosome, also support a recent African ancestry. However, the issue is not completely settled, because some types of genetic data can be interpreted in different ways under different assumptions of population structure and of the strength of natural selection acting on the traits. These differences illustrate the importance of gathering data on many different molecules, just as data need to be gathered on many morphological traits, when constructing phylogenies.

New Zealand

New Zealand

(a) Hypothesis 1: Single origin in Africa

Homo sapiens evolved from Homoerectus in Africa...

H. erectus

_ ^

Europe

Africa

Asia

...and migrated to other continents, where they displaced other Homo species.

(b) Hypothesis 2: Parallel origins in Europe, Africa, and Asia

H. erectus

Homo sapiens evolved simultaneously from *Homo erectus* populations in Africa, Europe, and Asia.

V



Europe

Africa

Asia

24.12 Two Models for the Origin of Modern Humans

There is considerable controversy among scientists as to whether the transition to *Homo sapiens* (red lineage) took place [a] only in Africa (hypothesis 1), or (b) occurred simultaneously on three continents (hypothesis 2). Current evidence from mtDNA and nuclear genes supports hypothesis 1.

*

MOLECULAR AND GENOMIC EVOLUTION 449

Chapter Summary

What Is Molecular Evolution?

- ▶ Molecular evolution differs from phenotypic evolution in that mutations and genetic drift are much more important determinants of rates of molecular evolution.
- ▶ The goals of the study of molecular evolution are to determine the patterns of evolutionary change in the molecules of which organisms are composed, to determine the processes that caused those changes, and to use those insights to help solve other biological problems.
- ▶ Neutral alleles are fixed slowly, whereas advantageous and disadvantageous alleles are fixed rapidly. Review Figure 24.1

Determining and Comparing the Structure of

Macromolecules

- ▶ The polymerase chain reaction method allows biologists to determine the nucleotide base sequences of organisms from their fossilized remains.
- ▶ Biological molecules can be compared by aligning their sequences. Review Figure 24.3
- ▶ Changes evolve slowly in regions of molecules that are functionally significant, but more rapidly in regions where base substitutions do not affect the functioning of the molecules. Review Figures 24.4, 24.5
- ▶ Rates of amino acid substitutions in some molecules are relatively constant over evolutionary time. Review Figure 24.6

Where Do New Genes Come From?

- ▶ Most new genes arise from gene duplication. The most important types of duplication are genome duplication (polyploidy) and domain duplication.
- ▶ Globin diversity evolved via gene duplication. Review Figure 24.7
- ▶ Groups of genes that are aligned in the same order on chromosomes of distantly related species are likely to be homologs of one another.

How Do Proteins Acquire New Functions?

- ▶ Changes in the functions performed by molecules may result from gene duplication if one gene retains the original function and the other evolves a new one.
- ▶ Homeotic genes have acquired varied functions in development.

Genome Organization and Evolution

- ▶ The genome sizes of organisms vary more than a hundredfold, but the amount of coding DNA varies much less. In general, eukaryotes have more coding DNA than do prokaryotes, vascular plants and invertebrate animals have more coding DNA

than do single-celled organisms, and vertebrates have more coding DNA than do invertebrates. Review Figures 24.9, 24.10

Using Biological Molecules to Reconstruct Phylogenetic Trees

► Biological molecules are an important source of data that can be used to infer phylogenetic relationships among organisms. For ancient splits and phylogenies of prokaryotes, molecular data are the only source of information about phylogenetic relationships.

► Molecules that have evolved slowly are useful for determining ancient lineage splits. Molecules that have evolved rapidly are useful for determining more recent lineage splits. Review Figure 24.11

Molecular Studies of Human Evolution

► Comparisons of mtDNA from more than 100 ethnically distinct modern human populations strongly suggest that all modern humans shared a common African ancestor no more than 200,000 years ago. Review Figure 24.12

For Discussion

1. If you were interested in reconstructing the phylogeny of a subgenus of fruit flies using molecular data, what kinds of molecule(s) would you choose to examine? Why? If you wanted to reconstruct the phylogeny of all vertebrates, would you use the same molecule(s)? Why or why not?
2. How have our views about organismal evolution been affected by recent applications of molecular methods to the study of evolution?
3. Discuss the relative importance of molecular characters versus morphological characters in reconstructing the phylogeny of a group of organisms.
4. Existing evidence suggests that for some molecules, a molecular clock ticks at a fairly constant rate, but that rates of change differ widely among molecules. How does this variation limit how and in what ways we can use the concept of a molecular clock to help us answer questions about the evolution of both molecules and organisms?
5. One hypothesis for the existence of large amounts of non-coding ("junk") DNA is that the cost of maintaining all that DNA is so small that natural selection is too weak to reduce it. What other hypotheses might account for the existence of so much noncoding DNA?
6. Why do reconstructions of the phylogenies of genes and phylogenies of the organisms that contain them often differ?
7. We are, by nature, interested in our own evolution. This chapter presented a brief introduction to the application of molecular methods to studying questions about human evolution. Make a short list of additional questions about human evolution and develop a rough outline of the molecules and methods you might bring to bear in addressing these questions.

7K

^ ^J The Origin of Life on Earth

Scientists believe that between 10 and 20 billion years ago there was a mighty explosion. The matter of the universe, which had been highly concentrated, began to spread apart rapidly. Eventually clouds of matter collapsed through gravitational attraction, forming the galaxies— great clusters of hundreds of billions of stars.

Somewhat less than 5 billion years ago, toward the outer edge of our galaxy (the Milky Way), our solar system (the sun, Earth, and our sister planets) took form. Earth probably formed about 4.5 billion years ago by gravitational attraction of rocks of various sizes. As Earth grew by this process, the weight of the outer layers compressed the interior of the planet. The resulting pressures, combined with energy from radioactive decay, heated the interior until it melted.

Within this viscous liquid, the heavier elements settled to produce a fluid iron and nickel core with a radius of approximately 3,700 km that persists to this day. Around the core lies a mantle of dense silicate material, called magma, that is 3,000 km thick. Over the mantle is a lighter crust, more than 40 km thick under the continents but as little as 5 km thick in some places under the oceans.

During the first half-billion years of its existence, Earth was bombarded by hundreds of rock bodies left over from the formation of the solar system. Collision with one of these bodies, which was at least as large as Mars, dislodged the material that became the moon. Many of these collisions were large enough to create a superheated atmosphere of vaporized rock that would have vaporized water and sterilized Earth's surface and subsurface.

Before the evolution of life, Earth's mantle and crust released carbon dioxide, nitrogen, and other heavier gases. These gases were held by Earth's gravitational field, and gradually, over several hundred million years, formed a new atmosphere consisting mostly of methane (CH₄), carbon dioxide (CO₂), ammonia (NH₃), hydrogen

The Big Bang

This computer-generated illustration of the Big Bang helps us visualize what that huge explosion might have looked like.

(H₂), nitrogen (N₂), and water vapor (H₂O). Eventually Earth cooled enough that the water vapor escaping from inside the planet condensed to liquid water and formed the oceans.

After Earth cooled enough for oceans to form and the bombardment was reduced to a very low level, life evolved on Earth. Fossils of complex unicellular life have been found in geological formations dated to 3.5 billion years ago (bya) (Figure 25.1). Scientists now believe that life first appeared on Earth about 4 billion years ago.

The first life must have come from nonliving matter. How did this happen? Under what conditions did life originate on Earth? This chapter describes how scientists try to answer these questions.

How Can We Study a Unique Event that Happened Several Billion Years Ago?

For the most part, scientists seek generalizations about nature. The most powerful scientific theories explain processes that occur repeatedly. Indeed, reproducibility is a key element of the hypothetico-deductive method. The scientific study of life's beginnings is different. The events leading to the origin of life may have happened only once, and we have no direct observational evidence of them. Since



?

I

O

«'• ■ 38\$'-'—"

r' '

25.1 The Oldest Traces of Life

Some of the oldest known fossils of microscopic unicellular life have been found in Australia. This 3.5 billion-year-old bacterium was found in Western Australia.

then, the state of Earth has been so altered that most of the traces of those early events have vanished. For this reason, the study of life's origins shares many characteristics with the study of history. It is inevitably more speculative than most components of scientific inquiry.

Nevertheless, three scientific principles can guide the study of the origin of life.

- ▶ The principle of continuity states that, because life probably evolved from nonlife by a continuous, gradual process, any stage in life's evolution that we propose should be derivable from preexisting states. In other words, we should not expect to find sudden major changes.
- ▶ The signature principle states that because of this historical continuity, prebiotic processes should leave some signatures—traces—in contemporary biochemistry.
- ▶ The third principle, which we may call the no-free-lunch principle, states that all living organisms require some form of energy for growth. More specifically, they must oxidize some material and obtain energy from that oxidation.

Using these three principles, scientists can focus their attention on hypotheses that are plausible, testable, and worthy of serious consideration.

To remind ourselves what it is that we must explain, let's briefly summarize the essential characteristics of life:

- ▶ All life is cellular.
- ▶ Life is based on aqueous solutions.

- ▶ The major atoms in all cells are carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur.
- ▶ Biochemical reactions take place inside cells.
- ▶ All proteins are made from the same group of amino acids, all RNA from the same group of ribonucleotides, and all DNA from the same group of deoxyribonucleotides.
- ▶ All carbohydrates are formed from a small group of sugars, and all phospholipids from a limited group of fatty acids.
- ▶ The flow of energy in the living world is accompanied by the formation and hydrolysis of phosphate bonds, usually those of ATP.
- ▶ All cells have an osmotically active barrier composed of lipids and associated proteins.
- ▶ The genome of every replicating cell is composed of DNA or RNA that is translated into polypeptides.
- ▶ All cells have ribosomes, and ribosomes are the sites of protein synthesis.
- ▶ All reactions that proceed rapidly in cells are catalyzed by proteins.
- ▶ Reproducing biological systems give rise to altered phenotypes as a result of mutated genotypes.

These basic chemical properties are the potential signatures of early life that can guide our study of life's origins. Their ubiquity shows that biochemical evolution has been remarkably conservative.

This conservatism helps us focus the scientific study of how life may have evolved from nonlife. For example, the earliest known molecular fossils were recently found in exceptionally well preserved shales from northwestern Australia, dated at 2.7 bya. These molecules are a type of lipid that is found today in the cell membranes of some photo-synthetic cyanobacteria.

Necessary Conditions for the Origin of Life

Living organisms are complex nonequilibrium systems that are maintained by the flow of usable energy—free energy (see Chapter 6). Disordered energy—entropy—cannot do work. Only two possible long-term sources of free energy for metabolism exist: radiation from the sun, and the chemical potential of reduced compounds in Earth's magma that are released when it flows to the surface. Both sources are used by organisms today. Either one, or both, could have powered the origin of life.

Conditions on early Earth differed from those of today

Free oxygen (O_2) probably was not present in Earth's early atmosphere. Any oxygen that was present reacted with hydrogen to form water, and with components of Earth's crust and atmosphere to form iron oxides, silicates, carbon dioxide, and carbon monoxide. Because oxygen was bound up with other elements, Earth had a reducing (electron-adding) atmosphere. As a setting for chemical reactions, then, early Earth differed fundamentally from present-day Earth, which has an oxidizing atmosphere containing large quantities of O_2 .

What sort of chemical reactions could have occurred in a reducing environment? Could such reactions have been the

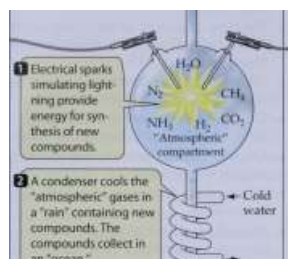
452 CHAPTER TWENTY-FIVE

EXPERIMENT

Question: Can organic compounds be generated under conditions similar to those that existed on primeval Earth?

METHOD

A solution of simple chemicals is heated, producing a reducing "atmosphere" of methane, ammonia, hydrogen, and water vapor.



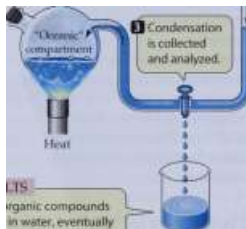
I Electrical sparks simulating lightning provide energy for synthesis of new compounds.

IA condenser cools the "atmospheric" gases in a "rain" containing new compounds. The compounds collect in an "ocean."

Cold water

Condensation-~

Q Condensation is collected and analyzed.



RESULTS

The organic compounds react in water, eventually forming purines, pyrimidines, and amino acids.

Conclusion: The organic building blocks of life are generated in the probable atmosphere of early Earth.

first step toward the origin of life? The first person to investigate these questions was Stanley Miller. In the 1950s, he established an experimental reducing atmosphere of hydrogen, ammonia, methane gas, and water vapor. Through these gases, he passed a spark to simulate lightning, then cooled the system so the gases would condense and collect in an aqueous solution, or "ocean" (Figure 25.2). Within hours, the system contained numerous simple organic compounds (compounds containing carbon, nitrogen, and hydrogen), including, for example, hydrogen cyanide and formaldehyde. Such compounds eventually reacted in water to form amino acids, purines, and pyrimidines— some of the building blocks of life.

The same or similar compounds can be produced under a variety of conditions, including ones that simulate conditions in aquatic environments, provided that free oxygen is



25.2 Synthesis of Prebiotic Molecules in an Experimental Atmosphere

Stanley Miller used an apparatus similar to this one to determine which molecules could be produced in a reducing atmosphere such as existed on early Earth.

absent. These findings suggest that once Earth cooled enough for water to condense and form oceans, molecules of many kinds probably formed. Over millions of years, these organic molecules would have accumulated in the oceans. They would have reached even higher concentrations in drying ponds or on the surfaces of clays.

Polymerization provided diverse macromolecules

The next stage in the sequence leading to life was the generation of large molecules by polymerization of small molecules. Polysaccharides, proteins, and nucleic acids are all polymers formed by the combination of subunits called monomers. As we saw in Chapter 3, polymers are assembled through repeated condensations of monomers. Each of these condensation reactions requires energy. Polymers that formed faster or were more stable would have come to predominate. High concentrations of polymers, in turn, would have stimulated further polymerization by shifting chemical equilibria from unstable monomers to more stable polymers.

Protobionts: Enclosing Prebiotic Systems

The experiments showing that a rich array of prebiotic molecules can be formed under the conditions likely to have existed on early Earth are highly informative, but a prebiotic soup of small molecules does not lead to life. For life to evolve, three additional conditions must be met:

- ▶ There must be a supply of replicators —molecules that are self-reproducing.
- ▶ The copying of these replicators must be subject to error via mutation.
- ▶ The system of replicators requires a perpetual supply of free energy and partial isolation from the general environment.

We will describe the source of replicators in the following sections. The requirement for mutation would have been easy to fulfill, because at the high temperatures found on early Earth, prebiotic molecules would have been continually altered as a result of thermal motion.

The evolution of membranes provided partial isolation

Partial isolation from the general environment can be achieved within aggregates of artificially produced prebiotic molecules. Called protobionts, these aggregates cannot reproduce, but they can maintain internal chemical environments that differ from their surroundings.

In the 1920s the Russian scientist Alexander Oparin observed that if he shook a mixture of a large protein and a polysaccharide, protobionts formed. Their interiors, which were primarily protein and polysaccharide, with some water, were separated from the surrounding aqueous solution, which had much lower concentrations of proteins and polysaccharides. Such protobionts, known as coacervates, are quite stable. They can be formed in solutions of many different types of polymers.

Oparin's coacervates also exhibited a simple form of metabolism. They absorbed substrates, catalyzed reactions, and let the products diffuse back into the aqueous solution (Figure 25.3). However, because these coacervates lacked lipid outer membranes, they differed from the probable precursors of life.

Other protobionts, called microspheres, form when mixtures of a variety of artificially produced organic compounds are mixed with cool water. If the mixture of compounds includes lipids, the surface of a microsphere consists of a lipid bilayer, similar to the lipid bilayer of cell membranes.

Membrane components became energy-transducing devices

Molecules that absorb visible or near-ultraviolet light— called chromophores —are likely to have been components of the lipid membranes of some protobionts. When light shines on protobionts that have chromophores in their membranes, electric potentials develop across the membranes. Such protobionts can become energy-transducing devices.

Given a continuous flux of light, oxidation-reduction reactions are possible if electrons can be conducted across the membrane. Acid-base-driven reactions are also possible if protons can be conducted across the membrane. These two types of reactions can be coupled because both are driven by the same electric potential.

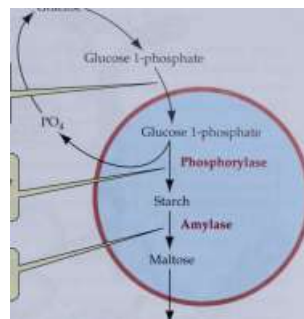
Today, the principal route of biological energy flow is from solar radiation to an oxidized and a reduced compound and the formation of some type of phosphate. The universality of this process suggests that it may have char-

Glucose

A coacervate drop absorbs glucose 1-phosphate from the surrounding medium.

Within the drop the enzyme phosphorylase polymerizes glucose to starch.

The enzyme amylase hydrolyzes starch to maltose.



Maltose

25.3 "Metabolism" of a Coacervate

The properties of artificial coacervates are similar to some of the properties of living cells. They are held intact by a nonlipid membranelike coating. Chemical reactions take place in the interior in the presence of enzymes.

acterized the earliest life and that protolife may have been driven primarily by solar energy.

RNA was probably the first biological catalyst

Some polymers can direct the synthesis of molecules identical to themselves. Which of the molecules on prebiotic Earth were most likely to reproduce themselves? The nucleic acids—the basis of today's genetic code—are good candidates. They are clearly capable of self-copying, and the purine and pyrimidine constituents of nucleotides were formed in Miller's experiment, under conditions similar to those believed to have prevailed on early Earth.

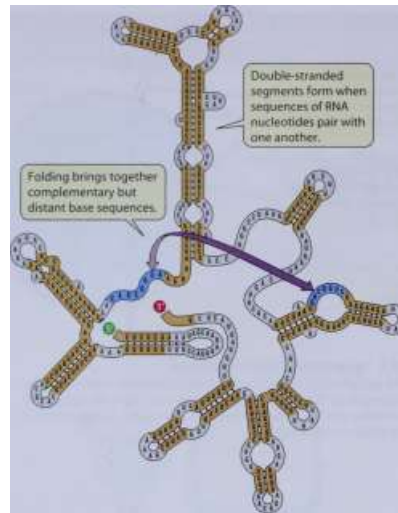
However, there is a problem with this idea. The enzymes that control the types and rates of reactions within organisms are proteins. As you learned in Chapter 12, proteins are synthesized by a process that begins with the transcription of information from DNA to mRNA. The information in mRNA is eventually used to synthesize a polypeptide from amino acids using another kind of RNA, tRNA. This system of protein synthesis (DNA → RNA → protein) probably evolved gradually from much simpler processes. How could such a system have evolved if proteins needed nucleic acids to form, but nucleic acids needed proteins to catalyze their replication? Which came first?

Inability to solve this dilemma held up research on the origin of life for several decades. The first clue came from experiments

in the late 1970s. When RNA molecules were added to solutions containing purines and pyrimidines, sequences of 5 to 10 nucleotides were formed. If a simple inorganic ion such as zinc was added, much longer sequences were copied.

454 CHAPTER TWENTY-FIVE

Folding brings together complementary but distant base sequences.



Double-stranded segments form when sequences of RNA nucleotides pair with one another.

25.4 A Ribozyme from a Protist

The folded three-dimensional structure of this catalytic RNA, or ribozyme, enables it to catalyze chemical reactions during protein synthesis. RNA catalysis may have preceded enzyme catalysis in the evolution of life.

eventually took over most enzymatic functions because they are better catalysts than RNA and are capable of more diverse specific activities.

To replicate, different RNA's would have competed with one another for monomers. Some RNA molecules would have been better at replicating in certain environments because their base sequences produced the most stable folded structures under the conditions of temperature and salinity they encountered. With their higher rates of replication and greater stability, these RNA molecules would have come to dominate the populations of RNA in their environments. Investigators have simulated the "evolution" of RNA molecules by selecting in test tubes for ribozymes with high catalytic ability. By this method they have produced ribozymes with reaction rates 7 million times faster than the uncatalyzed reaction rate, showing how highly catalytic RNA's might have evolved.

DNA evolved from an RNA template

If the first cells used RNA as their hereditary molecule, then RNA must have provided the template for the synthesis of DNA. In solution, DNA is less stable than RNA. Therefore, DNA probably did not evolve as a hereditary molecule until RNA-based life became enclosed in membranes within which water concentrations were lower than in the surrounding environment. In such cellular environments, DNA is a more stable storage molecule for genetic information than RNA. Therefore, once cells evolved, DNA probably rapidly replaced RNA as the genetic code for most organisms. But by then RNA's had assumed their current roles as intermediaries in the translation of genetic information into proteins.

The next discovery that provided a solution to the dilemma came in 1981 from scientists studying the excision of introns and the splicing together of exons. They found—entirely contrary to expectations—that these processes took place in the absence of enzymes! The intron itself—a 400-nucleotide sequence of RNA—catalyzed the excision and splicing.

In addition, it was discovered that ribosomes, which contain several molecules of RNA and a variety of proteins, have a catalytic RNA that operates in protein synthesis (see Chapter 12). RNA's that catalyze chemical reactions are called ribozymes (Figure 25.4).

Taken together, these discoveries suggest that the first genetic code was based on RNA that catalyzed its own replication as well as catalyzing other chemical reactions. A high concentration of RNA would have been needed so that it could participate in many different chemical reactions. The accumulated products of RNA-catalyzed reactions could then participate in other reactions and form structures. For example, RNAs could have catalyzed the formation of lipidlike molecules that could form

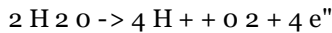
membranes, and of proteins that could catalyze the synthe-

; of other proteins. However, after proteins evolved, they

Photosynthesis Is the Source of Atmospheric O₂

The evolution of noncyclic photophosphorylation slightly more than 2 billion years ago changed the course of evolution and

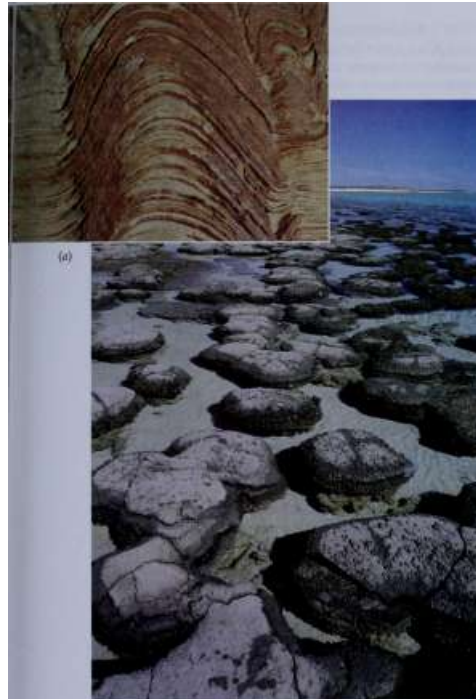
changed Earth. The key change was the ability of living organisms to use water as their source of hydrogen:



By chemically splitting H_2O , they generated O_2 as a waste product and made electrons available for reducing CO_2 to form organic compounds.

The ability to split water molecules appeared first in certain sulfur bacteria that evolved into cyanobacteria. Remains of these bacteria are abundantly fossilized in concentrations called stromatolites. Cyanobacteria are still forming stromatolites in a few very salty places on Earth (Figure 25.5).

Their water-splitting ability was doubtless the cause of the extraordinary success of cyanobacteria. The O_2 they liberated opened the way for the evolution of aerobic oxidation reactions as the energy source for the synthesis of ATP. Aerobic metabolism was much more rapid and efficient than the anaerobic metabolism that had dominated life



(b)

25.5 Stromatolites

(a) A vertical section through a fossil stromatolite, (b) These rocklike structures are living stromatolites that thrive in the very salty waters of Shark Bay, Western Australia. Layers of cyanobacteria are found in the uppermost parts of the structures.

until then. The success of the cyanobacteria made possible the evolution of the full respiratory chain of reactions now carried out by all aerobic cells.

The evolution of life irrevocably changed the nature of our planet. When it first appeared, oxygen was poisonous to the anaerobic organisms that were living on Earth at the time. Those prokaryotes that evolved a tolerance to O_2 were able to successfully colonize environments empty of other organisms and proliferate in great abundance. Life created the O_2 of our atmosphere, and it removed most of the carbon dioxide from the atmosphere by incorporating it into organic compounds and subsequently transferring it to ocean sediments.

Is Life Evolving from Nonlife Today?

Scientists have gathered information that provides many insights into the origin of life on Earth. Taken together, this information suggests that the evolution of life as we know it was highly probable under the conditions that prevailed on Earth 4 billion years ago. The molecules on which life is based form readily under such conditions, and those molecules readily organize themselves into larger units. Thus, the origin of life may have been almost inevitable.

However, new life apparently is not being assembled from nonliving matter on Earth today. Until the mid-1800s, people believed that life could arise by spontaneous generation from nonliving substances—for example, that frogs could arise from moist soil. The experiments that finally disproved the theory of spontaneous generation were performed in 1862 by the great French scientist Louis Pasteur. His experiments showed that microorganisms come only from other microorganisms and that a genuinely sterile solution remains lifeless indefinitely unless contaminated by living creatures (Figure 25.6). As a result of Pasteur's experiments and similar ones by other scientists, most people now accept that all life comes from existing life.

Why is it that new life is not being assembled from nonliving matter on today's Earth? The reason is that simple biological molecules released into today's environment are quickly consumed by existing life. They cannot accumulate to the densities

that characterized the "primordial soup," even in anaerobic environments. In aerobic environments, these molecules are quickly oxidized to other forms. Thus, they could not accumulate even if they were not consumed. Generation of life from nonlife on Earth did happen, but it was an event of the remote past. Once life had evolved, it prevented other life from arising from nonlife.

Does Life Exist Elsewhere in the Universe?

People have long speculated about the possibility of life on other planets in our solar system or in other solar systems. Recent evidence suggesting that life may exist on Mars or on one of the moons of Jupiter has fueled these speculations. Whether life exists or has existed on other planets in our solar system may be determined by future explorations of those planets. For other solar systems, we must rely on indirect methods. One approach is to identify the conditions on Earth that enabled life to evolve and that maintain life today.

Life was able to evolve on Earth because a set of conditions existed here that were suitable for the origin of single-celled organisms. For a planet to be able to support simple life, it must be associated with a star that has a relatively constant energy output and be far enough from it to be sufficiently cool for liquid water to form on its surface. These conditions are likely to be found in many places in the universe.

Multicellular organisms, on the other hand, are more exacting in their requirements. A more stable environment is

456 CHAPTER TWENTY-FIVE

EXPERIMENT

Question: Pasteur asked "Does life generate spontaneously or does it come only from already existing life"?

METHOD

Experiment 1

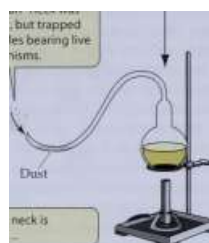
Experiment 2



Dust



A long "swan" neck was open to air, but trapped dust particles bearing live microorganisms.



If the swan neck is broken off...

RESULTS

... dust particles and live microorganisms enter the flask and grow rapidly in the rich nutrient medium.

Microbial growth



No microbial growth



Conclusion: All life comes from existing life.

ra



25.6 Experiments Disproved the Spontaneous Generation of Life

Louis Pasteur's classic experiments showed that, under today's conditions, a genuinely sterile solution remains lifeless indefinitely. Only after being "contaminated" by living organisms did life appear in the flasks.

required for their evolution and long-term survival. For multicellular life to evolve and survive, a planet must have other nearby planets large enough to intercept most of the comets and large meteors that would otherwise regularly

strike it and obliterate complex organisms. The planet also must have a nearly circular orbit and a rate of spin fast enough that extreme cold does not develop during the long night nor extreme heat during the long day. It also must have a moon large enough to dampen the planet's rotational irregularity.

These conditions result in a water-bathed environment in which temperature fluctuations, both diurnally and seasonally, are relatively small and the rate of occurrence of major perturbations that result in mass extinctions is low. The combination of these conditions is probably extremely rare in the universe. Therefore, even though microbial life may be widespread in the universe, Earth may be the only place, or one of only a few places, where multicellular life evolved and exists today. But the search for other planets that meet these stringent conditions is still in its infancy, and the universe of possibilities is very large.

Archaea and Bacteria had the planet to themselves for almost 3 billion years, and thousands of highly successful species of these prokaryotes exist today. The fossil record we discussed in Chapter 20 shows that although periods of mass extinction have occurred, conditions on Earth have been suitable for multicellular life for nearly a billion years. The result is that today Earth supports a rich and diverse array of species of both unicellular and multicellular organisms. The next section of this book is a brief overview of the many diverse forms life on Earth takes.

Chapter Summary

How Can We Study a Unique Event that Happened Several Billion Years Ago?

► Life originated from nonliving matter nearly 4 billion years ago. Even though the origin of life was a unique event, it can be studied scientifically by following three principles—the principle of continuity, the signature principle, and the "no-free-lunch" principle.

Necessary Conditions for the Origin of Life

► Conditions on Earth at the time of life's origin differed from those of today because Earth had a reducing atmosphere. Under conditions that resemble Earth's early atmosphere, small molecules essential to living systems form and polymerize. Review Figure 25.2

► Before life appeared, polymerization reactions generated the carbohydrates, lipids, amino acids, and nucleic acids of which organisms are composed. These molecules accumulated in the oceans.

Protobionts: Enclosing Prebiotic Systems

► The earliest protobionts probably had lipid-based membranes. Review Figure 25.3

► The first genetic material may have been RNA that had a catalytic function as well as an information transfer function. Some RNA's—called ribozymes—have catalytic functions today. Review Figure 25.4

► DNA probably evolved after RNA-based life became surrounded by membranes that provided an environment in which DNA was stable.

Photosynthesis Is the Source of Atmospheric O_2

► Cyanobacteria, which evolved the ability to split water into hydrogen ions and O_2 , proliferated and created atmospheric O_2

2. The accumulation of free O_2 in Earth's atmosphere made possible the evolution of aerobic metabolism.

Is Life Evolving from Nonlife Today?

- ▶ Because most of the chemical reactions that gave rise to life occur readily under the conditions that prevailed on the early Earth, life's evolution was probably nearly inevitable.
- ▶ Experiments by Louis Pasteur and others convinced scientists that life does not come from nonlife on Earth today. Review Figure 25.6
- ▶ New life is no longer being assembled from nonliving matter today because simple biological molecules that form in today's environment are quickly oxidized or consumed by existing life.

Does Life Exist Elsewhere in the Universe?

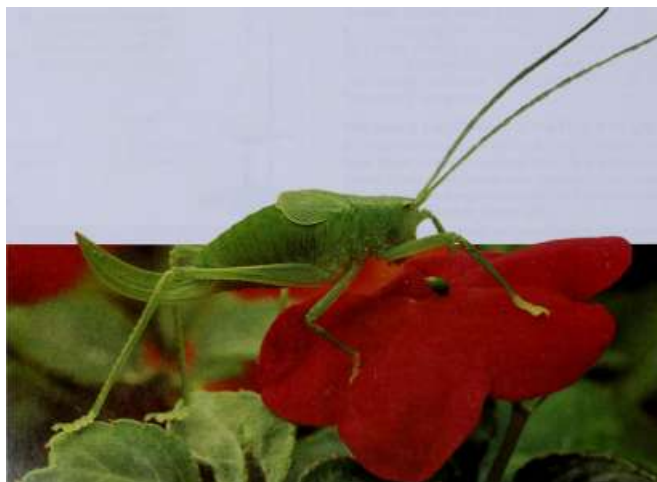
- ▶ The conditions that permit the evolution and maintenance of simple prokaryotic life may be widespread in the universe, but multicellular life has more stringent requirements, including a planet with a relatively circular orbit, a rapid rate of spin, nearby planets that intercept impacts, and a large moon that stabilizes the planet's orbit. Such conditions may be very rare.
- ▶ Although conditions on Earth have fluctuated greatly, they have been suitable for multicellular organisms for nearly a billion years.

For Discussion

1. Why is determining the composition of Earth's early atmosphere a key component of inferring how life arose?
2. Why is the ability of ribozymes to catalyze both their own synthesis and the synthesis of proteins so important for understanding the origin of life?
3. Scientists are confident that life no longer arises from nonliving matter under current conditions on Earth. Yet, biologists believe that life did arise on this planet, nearly 4 billion years ago, from nonliving matter. How can scientists hold both of these beliefs?
4. Why do biologists believe that the evolution of life was highly probable on early Earth?
5. How might each of the following have been involved in the evolution of coacervates?
 - a. Coating coacervate boundaries with lipids
 - b. Wave action in bodies of water
 - c. Catalysts within coacervates
6. Some people think that intelligent life exists on many planets in the universe. Others think that Earth may be the only planet where complex, multicellular life evolved. Which view do you support? Why?

Part Four

The Evolution of Diversity





A team of German scientists had found some organisms living in oceanic sediments off the coast of Chile. These organisms, too small to be seen except with a microscope, were able to grow and reproduce using sulfur present in the ooze of the ocean floor for their energy supply. Each was a bacterium, well known as being among the smallest of cells.

Now the oceanographers were looking for similar sulfur-using bacteria in sediments off the coast of Namibia in southwestern Africa. And they found them—but this time they didn't need a microscope. Strings of white bacteria, each the size of the period at the end of this sentence, were plainly visible to the naked eye. At up to 0.75 mm in diameter, these cells were the largest bacteria ever found. In comparison to typical bacteria, they were as large as a blue whale—the largest animal in the world—would be compared with a mouse.

How can single-celled bacteria be so different in size, yet carry out the same functions? A key in this case is that most of the huge bacterial cell (named *Thiomargarita namibiensis* by scientists) is filled with stored nitrate, which the cell uses to oxidize sulfur. But the "working chemistry" of *Thiomargarita* is remarkably similar to that of microscopic bacterial species.

Bacteria were first identified by the early microscopists some 300 years ago. Bacteria are prokaryotes, but they are not the only prokaryotes. The Archaea is a superficially similar group of microscopic, unicellular prokaryotes. Both the biochemistry and the genetics of bacteria differ in numerous ways from those of archaea. Not until the 1970s did biologists discover how radically different bacteria and archaea really are. And only with the sequencing of an archaean genome in 1996 did we realize just how extensively archaea differ from both bacteria and eukaryotes.

Many biologists acknowledge the antiquity of these lineages and the importance of their differences by recognizing three domains of living things: Bacteria, Archaea, and Eukarya. The domain Bacteria comprises the "true bacteria"; the domain Archaea (from the Greek *archaios*, "ancient") comprises other prokaryotes once called, inaccurately,

The Largest Known Bacterium

Three cells of the bacterium *Thiomargarita namibiensis*. The middle cell is about 0.2 mm in diameter; the scale bar at the left represents the size of a large "typical" bacterium, giving a sense of the size of this giant among its kind. The light dots are globules of sulfur.

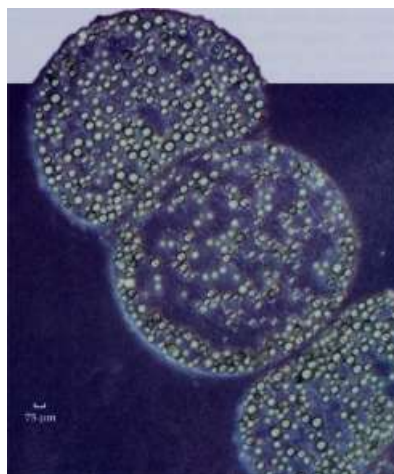
"ancient bacteria." The domain Eukarya comprises all other living things on Earth. Dividing the living world in this way, with two prokaryotic domains and a single domain for all the eukaryotes, fits with the current trend toward reflecting evolutionary relationships in classification systems.

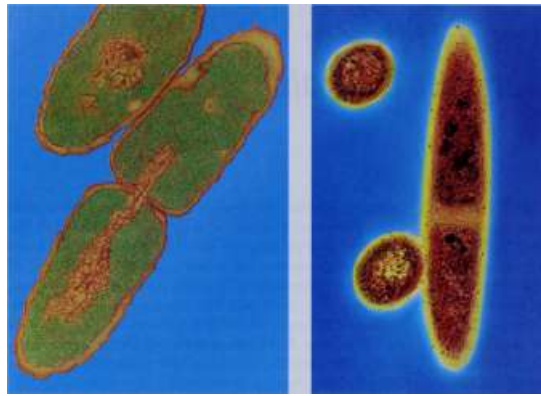
In the eight chapters of Part Four, we celebrate and describe the diversity of the living world—the products of evolution. This chapter focuses on the two prokaryotic domains. Chapters 27–33 deal with the protists and the kingdoms Plantae, Fungi, and Animalia.

In this chapter, we will pay close attention to the ways in which the two domains of prokaryotic organisms resemble each other, and how they differ. We will describe the impediments to the resolution of evolutionary relationships among the prokaryotes. Then we will survey the surprising diversity of organisms within each of the two domains, relating the characteristics of different prokaryotic groups to their roles in the biosphere and in our lives.

Why Three Domains?

What does it mean to be different? You and the person nearest you look very different—certainly you appear more dif-





Salmonella typhimurium

Methanospirillum hungatii

26.1

The Three Domains of Life on Earth

0.75 urn $i^{TMTM} > \ll \ll f/H \ll \cdot \gg H \gg \gg \gg / .j > \gg h 0.4 \text{ fun}$

26.1 Very Different Prokaryotes

In each image, one of the cells has nearly finished dividing. On the left are bacteria; on the right are archaea, which are more closely related to eukaryotic organisms than they are to the bacteria.

ferent than the two cells shown in Figure 26.1. But the two of you are members of the same species, and these two tiny organisms are classified in entirely separate domains. You (in the domain Eukarya) and those two prokaryotes (in the domains Bacteria and Archaea) have a lot in common. Members of all three domains

conduct glycolysis, and they , ^ ^ — ^ ^ ^ ^ ^

replicate their DNA semiconserv-atively. In all three, the DNA encodes polypeptides that are produced by transcription and translation, and the cells have plasma membranes and ribo-somes in abundance.

As a member of the domain Eukarya, you have cells with nuclei, membrane-enclosed organelles, and a cytoskeleton—things that no prokaryote has. However, a glance at Table 26.1 will show you that there are also major differences, most of which cannot be seen even under the microscope, between the two prokaryotic domains. In some ways the archaea are more like us; in other ways they are more like bacteria.

Genetic studies have led many biologists to conclude that all three domains had a single com-

mon ancestor and that the present-day archaea share a more recent common ancestor with eukaryotes than they do with bacteria (Figure 26.2). Because of the ancient time at which these three lineages diverged, the major differences among the three kinds of organisms, and especially the fact that the archaea are more closely related to the eukaryotes than are either of those groups to the bacteria, many biologists agree that it makes sense to treat these three groups as domains—a higher tax-onomic category than kingdoms. To treat all the prokaryotes as a single kingdom within a five-kingdom classification of organisms would result in a kingdom that is paraphyletic. That is, a single kingdom "Prokaryotes" would not include all the descendants of their common ancestor. (See Chapter 23, especially Figure 23.11, for a discussion of paraphyletic groups.) The domain concept is still controversial, and it may have to be abandoned if new data fail to support it. In this book, however, we will use the domain concept. The common ancestor of all three domains was prokaryotic. Its genetic material was DNA; common machinery for transcription and translation produced RNA's and proteins, respectively. It probably had a circular chromosome, and many of its structural genes were grouped into operons.

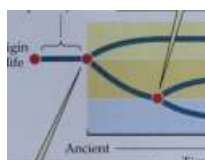
DOMAIN

" 70S ribosomes are smaller than 80S ribosomes.

Very ancient prokaryotes

Archaea and Eukarya are more closely related to each other than they are to Bacteria.

Origin of life



BACTERIA

ARCHAEA

EUKARYA

► Present

Time

Today's organisms all share this common ancestor.

26.2 The Three Domains of the Living World

Biologists believe that the three domains share a common pro-karyotic ancestor. The relationships shown here are controversial.

The Archaea, Bacteria, and Eukarya of today are all the products of billions of years of natural selection and genetic drift, and they are all highly adapted to present-day environments. None are "primitive." The common ancestor of the Archaea and the Eukarya probably lived more than 2 billion years ago, and the common ancestor of the Archaea, the Eukarya, and the Bacteria probably lived more than 3 billion years ago.

The earliest prokaryotic fossils date back at least 3.5 billion years, as we saw in Chapter 25, and these ancient fossils indicate that there was considerable diversity among the prokaryotes even during the earliest days of life. The prokaryotes were alone on Earth for a very long time, adapting to new environments and to changes in existing environments.

General Biology of the Prokaryotes

There are many, many prokaryotes around us—everywhere. Although most are so small that we cannot see them with the naked eye, the prokaryotes are the most successful of all creatures on Earth, if success is measured by numbers of individuals. The bacteria in one person's intestinal tract, for example, outnumber all the humans who have ever lived, and even the total number of human cells in that person's body. Some of these bacteria form a thick lining along the intestinal wall.

Although small, prokaryotes play many critical roles in the biosphere, interacting in one way or another with every

BACTERIA AND ARCHAEA: THE PROKARYOTIC DOMAINS 461

other living thing. In this section on the general biology of the prokaryotes, we'll see that some perform key steps in the cycling of nitrogen, sulfur, and carbon. Other prokaryotes trap energy from the sun or from inorganic chemical sources, and some help animals digest their food. The members of the two prokaryotic domains outdo all other groups in metabolic diversity. Eukaryotes, in contrast, are much more diverse in size and shape, but their metabolism is much less diverse. In fact, much of the energy metabolism of eukaryotes is carried out in organelles—mitochondria and chloroplasts—that are descended from bacteria.

Prokaryotes are found in every conceivable habitat on the planet, from the coldest to the hottest, from the most acidic to the most alkaline, and to the saltiest. Some live where oxygen is abundant and others where there is no oxygen at all. They have established themselves at the bottom of the seas, in rocks more than 2 km into Earth's solid crust, and even inside other organisms, large and small. Their effects on our environment are diverse and profound.

Prokaryotes and their associations take a few characteristic forms

Three shapes are particularly common among the prokaryotes: spheres, rods, and curved or spiral forms (Figure 26.3). A spherical prokaryote is called a coccus (plural cocci). Cocci may live singly or may associate in two- or three-dimensional arrays as chains, plates, or blocks of cells. A rod-shaped prokaryote is called a bacillus (plural bacilli). Bacilli and spiral forms, the third main prokaryotic shape, may be single or may form chains.

Prokaryotes are almost all unicellular, although some multicellular ones are known. Associations such as chains do not signify multicellularity, because each cell is fully viable and independent. Associations arise as cells adhere to one another after reproducing by fission. Some bacteria associate in chains that become enclosed within delicate tubular sheaths. These associations are called filaments. All the cells of a filament divide simultaneously.

26.3 Shapes of Prokaryotic Cells

(a) These spherical cocci of an acid-producing bacterium grow in the mammalian gut. (b) Rod-shaped *E. coli* are the most thoroughly studied of all bacteria—indeed, of almost any organism on Earth, (c) A freshwater spiral bacteria species. The cells move by means of the tufts of flagella at each pole.



(a) *Enterococcus* sp.

1 (b) *Escherichia coli*

1 |im

■ (c) *Aquaspirillum sinuatum*

462 CHAPTER TWENTY-SIX

Prokaryotes lack nuclei, organelles, and a cytoskeleton

The architectures of prokaryotic and eukaryotic cells were compared in Chapter 4. The basic unit of archaea and bacteria is the prokaryotic cell (see Figure 4.5), which contains a full complement of genetic and protein-synthesizing systems, including DNA, RNA, and all the enzymes needed to transcribe and translate the genetic information into proteins. The prokaryotic cell also contains at least one system for generating the ATP it needs.

In what follows, bear in mind that most of what we know about the structure of prokaryotes comes from studies of bacteria. We still know relatively little about the diversity of archaea, although the pace of research on archaea is accelerating.

The prokaryotic cell differs from the eukaryotic cell in three important ways. First, the organization and replication of the genetic material differs. The DNA of the prokaryotic cell is not organized within a membrane-enclosed nucleus. DNA molecules in prokaryotes are usually circular; in the best-studied prokaryotes, there is a single chromosome, but there are often plasmids as well (see Chapter 13).

Second, prokaryotes have none of the membrane-enclosed cytoplasmic organelles that modern eukaryotes have—mitochondria, Golgi apparatus, and others. However, the cytoplasm of a prokaryotic cell may contain a variety of infoldings of the plasma membrane (see Figure 4.6) and photosynthetic membrane systems not found in eukaryotes. Membranous infoldings frequently associate with new cell walls during cell division. In electron micrographs, the DNA of a bacterial cell is often seen attached to such an infolding, called a mesosome (Figure 26.4).

(«)

Internal fibrils (axial filaments)

The mesosome in this bacterium is continuous with the plasma membrane.

The cell's DNA is attached to the mesosome.



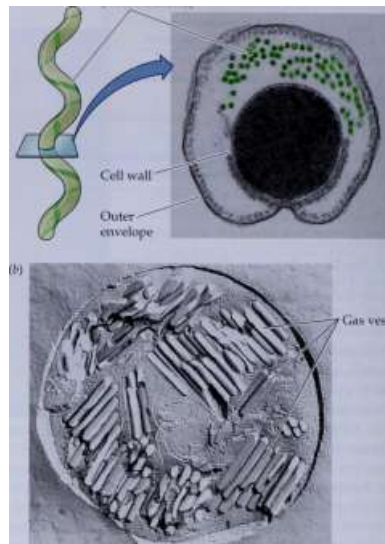
Mesosome

Plasma membrane

Corynebacterium parvum

26.4 Some Prokaryotes Have Internal Membranes

Unlike eukaryotic organelles, the mesosome in this bacterial cell is not a separate, membrane-enclosed compartment.



Gas vesicles

26.5 Structures Associated with Prokaryote Motility

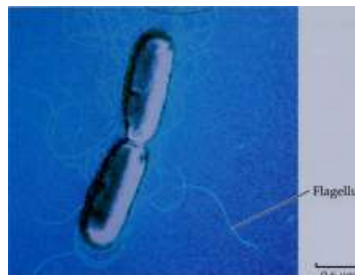
(a) A spirochete from the gut of a termite, seen in cross section, shows the fibrils used to produce a rolling motion, (b) Gas vesicles in a cyanobacterium, visualized by the freeze-etch technique.

Third, prokaryotic cells lack a cytoskeleton, and, without the cytoskeletal proteins, they lack mitosis. Prokaryotic cells divide by their own elaborate method, fission, after replicating their DNA.

Prokaryotes have distinctive modes of locomotion

Although many prokaryotes are not motile, others can move by one of several means. Some spiral bacteria called spirochetes use a rolling motion made possible by internal fibrils (Figure 26.5a). Many cyanobacteria and some other bacteria use various poorly understood gliding mechanisms, including rolling. Some aquatic prokaryotes, including some cyanobacteria, can move slowly up and down in the water by adjusting the amount of gas in gas vesicles (Figure 26.5b). By far the most common type of locomotion in prokaryotes is that driven by flagella.

Bacterial flagella are whiplike filaments that extend singly or in tufts from one or both ends of the cell (see Figure 26.3c and 26.8f), or all around it (Figure 26.6). A bacterial flagellum consists of a single fibril made of the protein flagellin, projecting from the cell surface (see Figure 4.7). In contrast, the flagellum of eukaryotes is enclosed by the plasma membrane and usually contains a circle of nine pairs of microtubules surrounding two central micro-



Flagellum

0.6 μm

26.6 Some Bacteria Use Flagella for Locomotion

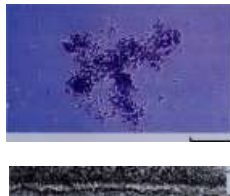
Flagella surround the rod-shaped cells of this *Bacillus* species.

tubules, all containing the protein tubulin, along with many other, associated proteins. The prokaryotic flagellum rotates about its base, rather than beating, as a eukaryotic flagellum or cilium does.

Prokaryotes have distinctive cell walls

Most prokaryotes have a thick and relatively stiff cell wall. This wall is quite different from the cell walls of plants and algae, which contain cellulose and other polysaccharides, and of fungi, which contain chitin. Almost all bacteria have cell walls containing peptidoglycan (a polymer of amino sugars). Archaeal cell walls are of differing types, but most contain significant amounts of protein. One group of archaea has pseudopeptidoglycan in its wall; as you have probably already guessed from the prefix pseudo-, pseudopeptidoglycan is similar to, but distinct from, the peptidoglycan of bacteria. Peptidoglycan is a substance unique to

(a) *Bacillus subtilis*



bacteria; its absence from the walls of archaea indicates a key difference between the two prokaryotic domains.

In 1884 Hans Christian Gram, a Danish physician, developed a simple staining process that has lasted into our high-technology era as the single most common tool in the identification of bacteria. The Gram stain separates most types of bacteria into two distinct groups, Gram-positive and Gram-negative, on the basis of their cell wall structure (Figure 26.7). A smear of cells on a microscope slide is soaked in a violet dye and treated with iodine; it is then washed with alcohol and counterstained with safranine (a red dye). Gram-positive bacteria retain the violet dye and appear blue to purple (Figure 26.7a). The alcohol washes the violet stain out of Gram-negative cells; these cells then pick up the safranine counterstain and appear pink to red (Figure 26.7b). Gram-staining characteristics are a crucial consideration in classifying some kinds of bacteria and are important in determining the identity of bacteria in an unknown sample. Mycoplasmas, which lack cell walls, are not stained at all by the Gram stain.

For the majority of the bacteria, the Gram-staining results correlate with the structure of the cell wall. Peptidoglycan forms a thick layer outside the plasma membrane of Gram-positive bacteria. The Gram-negative cell wall usually has only one-fifth as much peptidoglycan, and outside the peptidoglycan layer the cell is surrounded by a second, outer membrane quite distinct in chemical makeup from the plasma membrane (see Figure 26.7b). The space between the inner (plasma) and outer membranes of Gram-negative bacteria is called the periplasmic space. The peri-plasmic space contains enzymes that are important in

26.7 The Gram Stain and the Cell Wall

When treated with a Gram stain, the cell wall components of different bacteria react in one of two ways, (a) Gram-positive bacteria retain the violet dye and appear deep blue or purple; the pink counterstain surrounds the cells in this micrograph, (b) Gram-negative bacteria do not retain the violet dye but are made visible on the slide by the counterstain and appear pink-red.

Peptidoglycan Plasma membrane

Cytoplasm



(b) *Escherichia coli*

10 urn

60 nm

. *WA*)* ST.

Capsule Outer membrane

Peptidoglycan

- ; .-' ■•:? Plasma membrane

-.

Cytoplasm



5 nm

60 nm

digesting some materials, transporting others, and detecting chemical gradients in the environment

The consequences of the different features of prokaryotic cell walls are numerous and relate to the disease-causing characteristics of some prokaryotes. Indeed, the cell wall is a favorite target in medical combat against diseases that are caused by prokaryotes, because it has no counterpart in eukaryotic cells. Antibiotics and other agents that specifically interfere with the synthesis of peptidoglycan-containing cell walls tend to have little, if any, effect on the cells of humans and other eukaryotes.

Prokaryotes reproduce asexually, but genetic recombination does occur

Prokaryotes reproduce by fission, an asexual process. Recall, however, that there are also processes—transformation, conjugation, and transduction—that allow the exchange of genetic information between some prokaryotes quite apart from either sex or reproduction (see Chapter 13).

Many prokaryotes multiply very rapidly. One of the fastest is the bacterium *Escherichia coli*, which under optimal conditions has a generation time of about 20 minutes. The shortest known prokaryote generation times are about 10 minutes. Values of 1 to 3 hours are common; some extend to days. Bacteria living in rock deep in Earth's crust may suspend their growth for more than a century without dividing and then grow for a few days before suspending growth again.

Prokaryotes have exploited many metabolic possibilities

The long evolutionary history of the bacteria and archaea, including their explorations of new environments, has led to the extraordinary diversity of their metabolic "lifestyles"—their use or nonuse of oxygen, their energy sources, the sources of their carbon atoms, and the materials they secrete.

ANAEROBIC VERSUS AEROBIC METABOLISM. Some prokary-

otes can live only by anaerobic metabolism because oxygen gas is poisonous to them. These oxygen-sensitive organisms are called obligate anaerobes.

Other organisms can shift their metabolism between anaerobic and aerobic modes (see Chapter 7) and thus are called facultative anaerobes. Some facultative anaerobes cannot conduct cellular respiration, but are not damaged by oxygen when it is present. Many prokaryotes are facultative anaerobes that alternate between anaerobic metabolism (such as fermentation) and cellular respiration as conditions dictate.

At the other extreme from the obligate anaerobes, some prokaryotes are obligate aerobes, unable to survive for extended periods in the absence of oxygen.

nutritional categories. Biologists recognize four broad nutritional categories of organisms: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs. Prokaryotes are represented in all four groups (Table 26.2).

Photoautotrophs are photosynthetic. They use light as their source of energy and carbon dioxide as their source of carbon. Like the photosynthetic eukaryotes, the cyanobacteria, one group of photoautotrophic bacteria, use chlorophyll *a* as their key photosynthetic pigment and produce oxygen as a by-product of noncyclic photophosphorylation (see Chapter 8).

By contrast, the other photosynthetic bacteria use bacteriochlorophyll as their key photosynthetic pigment, and they do not release oxygen gas. Some of these photosynthesizers produce particles of pure sulfur instead because hydrogen sulfide (H_2S), rather than H_2O , is their electron donor for photophosphorylation (Figure 26.8a). Bacteriochlorophyll absorbs light of longer wavelengths than the chlorophyll used by all other photosynthesizing organisms does. As a result, bacteria using this pigment can grow in water beneath fairly dense layers of algae, using light of wavelengths that are not appreciably absorbed by the algae (Figure 26.8b).

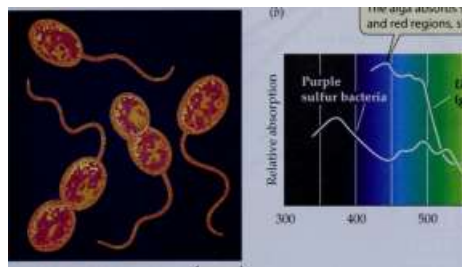
Photoheterotrophs use light as their source of energy, but must obtain their carbon atoms from organic compounds made by other organisms. They use compounds such as carbohydrates, fatty acids, and alcohols as their organic "food." The purple nonsulfur bacteria, among others, are photoheterotrophs.

Chemoautotrophs obtain their energy by oxidizing inorganic substances, and they use some of that energy to fix carbon dioxide. Some chemoautotrophs use reactions identical to those of the photosynthetic carbon reduction cycle (see Chapter 8), but others use other pathways to fix carbon dioxide. Some bacteria oxidize ammonia or nitrite ions to form nitrate ions. Others oxidize hydrogen gas, hydrogen sulfide, sulfur, and other materials. Some archaea are chemoautotrophs (Figure 26.9).

Some deep-sea ecosystems are based on chemoauto-trophic prokaryotes that are incorporated into large com-

BACTERIA AND ARCHAEA: THE PROKARYOTIC DOMAINS 465

The alga absorbs strongly in the blue and red regions, shading the bacteria.



(a) *Thiocystis* sp.

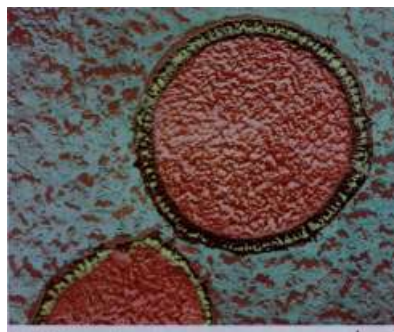
0.8 μ m

26.8 Some Bacteria are Photosynthetic

(a) Cells of purple sulfur bacteria store granules of sulfur that they produce via anaerobic photosynthesis, (b) *Ulva*, a green alga, absorbs no light of wavelengths longer than 750 nm. Purple sulfur bacteria can conduct photosynthesis using the longer wavelengths that pass through the algae.

communities of crabs, mollusks, and giant worms, all living in near-boiling water at a depth of 2,500 meters, below any hint of light from the sun, but in the immediate neighborhood of volcanic vents in the ocean floor. These bacteria obtain energy by oxidizing hydrogen sulfide and other substances released from the vents.

Finally, chemoheterotrophs obtain both energy and carbon atoms from one or more complex organic compounds.



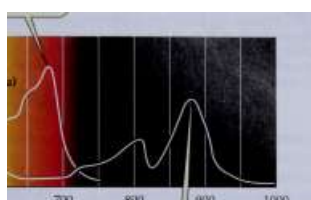
Staphylothermus marinus

0.2 μ m

26.9 Chemoautotrophs in Hot Water

These archaea are chemoautotrophs that live in the extremely hot water surrounding deep-sea volcanic vents. Chemoautotrophic archaea and bacteria fix carbon and support the nutritional needs of entire communities of hydrothermal vent organisms that thrive far below the reach of sunlight.

va sp. een alga)

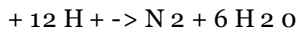
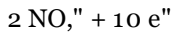


Wavelength (run)

Purple sulfur bacteria can use long-wavelength light, which the algae do not absorb, for their photosynthesis.

Most known bacteria and archaea are chemoheterotrophs— as are all animals and fungi, and many protists.

Nitrogen and sulfur metabolism. Some bacteria carry out respiratory electron transport without using oxygen as an electron acceptor. These forms use oxidized inorganic ions such as nitrate, nitrite, or sulfate as electron acceptors. Among these organisms are the ocean-dwelling bacteria mentioned at the beginning of this chapter. Other examples include the denitrifiers, bacteria that return nitrogen to the atmosphere as nitrogen gas (N_2), completing the cycle of nitrogen in nature. These normally aerobic bacteria, mostly species of the genera *Bacillus* and *Pseudomonas*, use nitrate (NO_3^-) in place of oxygen if they are kept under anaerobic conditions:



Nitrogen fixers convert atmospheric nitrogen gas into chemical forms usable by the nitrogen fixers themselves and by other living things. Some, for example, convert nitrogen gas to ammonia:



All organisms require nitrogen for their proteins, nucleic acids, and other important compounds. The vital process of nitrogen fixation is carried out by a wide variety of bacteria, including cyanobacteria, but by no other organisms. We'll discuss this process in detail in Chapter 36.

Ammonia is oxidized to nitrate by the process of nitrification. This process is carried out in the soil by chemoautotrophic bacteria called nitrifiers. Bacteria of two genera, *Nitrosomonas* and *Nitrosococcus*, convert ammonia to nitrite ions (NO_2^-), and *Nitrobacter* oxidizes nitrite to nitrate (NO_3^-). What do the nitrifiers get out of these reactions? Their chemosynthesis is powered by the energy released by oxidation of ammonia or nitrite. For example, by passing the electrons from nitrite through an electron transport chain, *Nitrobacter* can make ATP, and using some of this ATP, it can also make NADH. With the ATP and NADH, the

466 CHAPTER TWENTY-SIX



\n\n. 26.10 The Nitrogen Cycle

Bacteria carry out key steps in the cycling of nitrogen through the biosphere. Bacteria trap nitrogen gas (nitrogen fixation), convert the product to nitrate ions (nitrification), and return nitrogen gas to the atmosphere (denitrification) in the final step. Plants provide the nitrate reduction steps.

DENITRIFICATION

bacterium can convert CO_2 and H_2O to glucose and other foods. The nitrifiers base their entire biochemistry— their entire lives—on the oxidation of ammonia or nitrite ions.

Numerous bacteria base their metabolism on the modification of sulfur-containing ions and compounds in their environments. As examples, we have already mentioned the photoautotrophic bacteria and chemoautotrophic archaea that use H_2S as an electron donor in place of H_2O . Such uses of nitrogen and sulfur have obvious environmental implications, as we'll see in the next section.

Prokaryotes in Their Environments

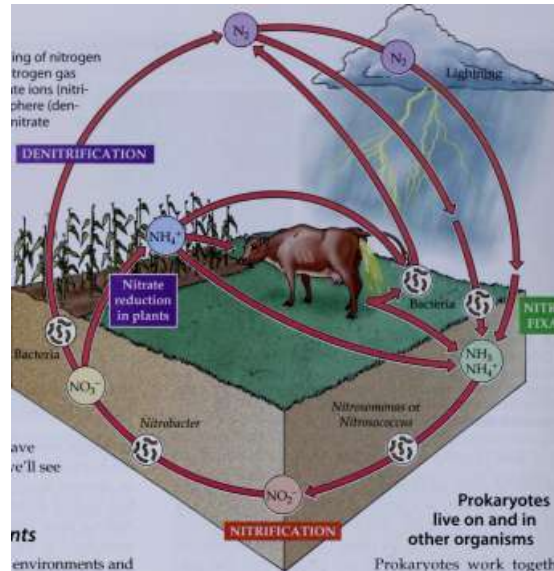
Prokaryotes live in and exploit all sorts of environments and are parts of many ecosystems. In the following pages, we'll examine prokaryotes living in soils, in water, and even in other living things, where they may exist in neutral, benevolent, or parasitic relationship with the host's tissues.

Prokaryotes are important players in element cycling

Animals depend on photosynthetic plants for their food, directly or indirectly. But plants depend on other organisms— prokaryotes—for their own nutrition. The extent and diversity of life on Earth would not be possible without biological nitrogen fixation (Figure 26.10). Nitrifiers are also crucial to the biosphere, because they convert the products of nitrogen fixation into nitrate ions, the form of nitrogen most easily used by many plants. Plants, in turn, are the source of nitrogen compounds for animals and fungi. Denitrifiers also play a key role in keeping the nitrogen cycle going. Without denitrifiers, which convert nitrate ions back into nitrogen gas, all forms of nitrogen would leach from the soil and end up in lakes and oceans, making life on land impossible. Other prokaryotes contribute to a similar cycle for sulfur.

In the ancient past, the cyanobacteria had an equally dramatic impact on life: Their photosynthesis generated oxygen, converting Earth from an anaerobic to an aerobic environment. The result was the wholesale loss of species that couldn't tolerate the O_2 generated by the cyanobacteria, but this transformation made possible the evolution of cellular respiration

and the subsequent explosion of eu-karyotic life.



NITROGEN FIXATION

Prokaryotes live on and in other organisms

Prokaryotes work together with eukaryotes in many ways. In fact, mitochondria and chloroplasts are descended from what were once free-living bacteria. Much later in evolutionary history, some plants formed associations with bacteria to form cooperative nitrogen-fixing nodules on their roots (see Chapter 36).

Many animals, including humans, harbor a variety of bacteria and archaea in their digestive tracts. Cows depend on prokaryotes to perform important steps in digestion. Like most animals, cows cannot produce cellulase, the enzyme needed to start the digestion of the cellulose that makes up the bulk of their plant food. However, bacteria living in a special section of the gut called the rumen produce enough cellulase to process the cow's daily diet. Humans use some of the metabolic products—especially vitamins B 12 and K—of bacteria living in our large intestine.

We are heavily populated, inside and out, by bacteria. Although very few of them are agents of disease, popular notions of bacteria as "germs" arouse our curiosity about those few. Let's briefly consider the roles of some bacteria as pathogens.

A small minority of bacteria are pathogens

The late nineteenth century was a productive era in the history of medicine—a time during which bacteriologists, chemists, and physicians proved that many diseases are caused by microbial agents. During this time the German physician Robert Koch laid down a set of rules for establishing that a particular microorganism causes a particular disease:

BACTERIA AND ARCHAEA: THE PROKARYOTIC DOMAINS 467

- ▶ The microorganism must always be found in individuals with the disease.
- ▶ The microorganism can be taken from the host and grown in pure culture.
- ▶ A sample of the culture produces the disease when injected into a new, healthy host.
- ▶ The newly infected host yields a new, pure culture of microorganisms identical to those obtained in the second step.

These rules—called Koch's postulates—were very important in a time when it was not widely accepted that microorganisms cause disease. Today, medical science makes use of other, more powerful diagnostic tools.

Only a tiny percentage of all prokaryotes are pathogens (disease-producing organisms), and of those that are known, all are bacteria. For an organism to be a successful pathogen, it must overcome several hurdles:

- ▶ It must arrive at the body surface of a potential host.
- ▶ It must enter the host's body.
- ▶ It must evade the host's defenses.
- ▶ It must multiply inside the host.
- ▶ It must infect a new host.

Failure to overcome any of these hurdles ends the reproductive career of a pathogenic organism. However, in spite of the many defenses available to potential hosts that we considered in Chapter 19, some bacteria are very successful pathogens.

For the host, the consequences of a bacterial infection depend on several factors. One is the invasiveness of the pathogen—its ability to multiply within the body of the host. Another is its toxigenicity—its ability to produce chemical substances (toxins) harmful to the tissues of the host. *Corynebacterium diphtheriae*, the agent that causes diphtheria, has low invasiveness and multiplies only in the throat, but its toxigenicity is so great that the entire body is affected. In contrast, *Bacillus anthracis*, which causes anthrax (a disease primarily of cattle and sheep), has low toxigenicity but an invasiveness so great that the entire bloodstream ultimately teems with the bacteria.

There are two general types of bacterial toxins: exotoxins and endotoxins. Endotoxins are released when certain Gram-negative bacteria lyse (burst). These toxins are lipo-polysaccharides that form part of the outer bacterial membrane. Endotoxins are rarely fatal; they normally cause fever, vomiting, and diarrhea. Among the endotoxin producers are some strains of *Salmonella* and *Escherichia*.

Exotoxins are proteins released by living, multiplying bacteria, and they may travel throughout the host's body. They are highly toxic—often fatal—to the host, but do not produce fevers. Exotoxin-induced human diseases include tetanus (from *Clostridium tetani*), botulism (from *Clostridium Botulinum*), cholera (from *Vibrio cholerae*) and plague (from *Yersinia pestis*).

Remember that in spite of our frequent mention of human pathogens, only a small minority of the known prokaryotic species are pathogenic. Many more species play

positive roles in our lives and in the biosphere. We make direct use of many bacteria and a few archaea in such diverse applications as cheese production, sewage treatment, and the industrial production of an amazing variety of antibiotics, vitamins, organic solvents, and other chemicals.

Prokaryote Phylogeny and Diversity

The prokaryotes comprise a diverse array of microscopic organisms. To explore this diversity, let's first consider how they are classified, and some of the difficulties involved in doing so; then we'll look at some specific examples.

Nucleotide sequences of prokaryotes reveal their evolutionary relationships

There are three primary motivations for classification schemes: to help identify unknown organisms, to reveal evolutionary relationships, and to provide universal names (see Chapter 23). Many scientists and medical technologists must be able to identify bacteria quickly and accurately—when the bacteria are pathogenic, lives may depend on it.

Until recently, taxonomists based their classification schemes for the prokaryotes on readily observable phenotypic characters such as color, motility, nutritional requirements, antibiotic sensitivity, and reaction to the Gram stain. Although such schemes have facilitated the identification of prokaryotes, they have not provided insights into how these organisms evolved—a question of great interest to microbiologists and to all students of evolution. The prokaryotes and the protists (see Chapter 27) have long been major challenges to those who attempted phylogenetic classifications. Only recently have systematists had the right tools for tackling this task.

Analyses of the nucleotide sequences of ribosomal RNA's provided us with the first apparently reliable measures of evolutionary distance among taxonomic groups. Ribosomal RNA (rRNA) is particularly useful for evolutionary studies of living organisms for several reasons:

- ▶ rRNA is evolutionarily ancient.
- ▶ No living organism lacks rRNA.
- ▶ rRNA plays the same role in translation in all organisms.
- ▶ rRNA has evolved slowly enough that sequence similarities between groups of organisms are easily found.

Let's look at just one of the approaches to the use of rRNA for studying evolutionary relationships.

Comparisons of rRNA's from a great many organisms showed that there are recognizable short base sequences characteristic of particular taxonomic groups. These signature sequences, approximately 6 to 14 bases long, appear at the same approximate positions in rRNA's from related groups. For example, the signature sequence AAACU-UAAAG occurs about 910 bases from one end of the RNA of the light subunit of ribosomes in 100 percent of the Archaea and Eukarya tested, but in none of the Bacteria tested. Several signature sequences distinguish each of the three domains. Similarly, the major groups within the bacteria and archaea possess unique signature sequences.

468 CHAPTER TWENTY-SIX



Common ancestor of all of today's organisms

These data sound promising, but things aren't as easy as we might wish. When biologists examined other genes and RNA's, contradictions began to appear. Analyses of different nucleotide sequences suggested different phylogenetic patterns. How could such a situation have arisen?

Lateral gene transfer muddied the phylogenetic waters

It is now clear that, from early in evolution to the present day, genes have been moving among prokaryotic species by lateral gene transfer. As we have seen, a gene from one species can become incorporated into the genome of another. Mechanisms of lateral gene transfer include transfer by plasmids and viruses and uptake of DNA by transformation. Such transfers are well documented, not just between bacterial species or archaean species, but also across the boundary between bacteria and archaea.

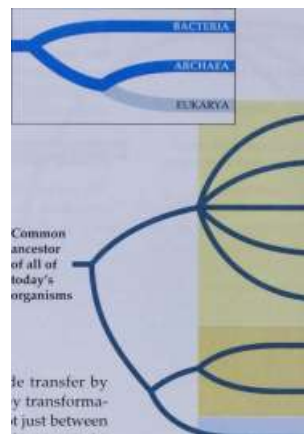
A gene that has been transferred will be inherited by the recipient's progeny and in time will be recognized as part of the normal genome of the descendants. Biologists are still assessing the extent of lateral gene transfer among prokaryotes and its implications for phylogeny, especially at the early stages of evolution.

There is great controversy now over prokaryotic phylogeny. Figure 26.11 is an overview of some major groups in the domains Bacteria and Archaea that we will discuss further in this chapter. Keep in mind that a new picture will likely emerge within the next decade, based on the addition of more nucleotide sequence data and on new information about the currently understudied archaea.

Mutations are the most important source of prokaryotic variation

Assuming that the prokaryote groups we are about to describe do indeed represent monophyletic groups, we discover that they are amazingly complex. A single group of bacteria or archaea may contain the most extraordinarily diverse species, and a species in one group may be phenotypically almost indistinguishable from one or many species in another group. What are the sources of this diversity?

Although prokaryotes can acquire new alleles by transformation, transduction, or conjugation, the most important source of genetic variation in populations of prokaryotes is probably mutation. Mutations, especially recessive mutations, are slow to make their presence felt in populations of humans and other diploid organisms. In contrast, a mutation in a prokaryote, which is haploid, has immediate consequences for that organism. If it is not lethal, it will be transmitted to and expressed in the organism's daughter cells—and in their daughter cells, and so forth. Thus, a beneficial mutant allele spreads rapidly.



Domains

Proteobacteria

BACTERIA

Cyanobacteria

Spirochetes

Chlamydias

Firmicutes

ARCHAEA

Crenarchaeota

Euryarchaeota

EUKARYA

Eukaryotes

26.11 Two Domains: A Brief Overview

An abridged summary classification of the domains Bacteria and Archaea shows their relationships to each other and to the Eukarya. The relationships among the many lineages of bacteria, not all of which are listed here, are unresolved at this time.

The rapid multiplication of many prokaryotes, coupled with mutation, selection, and genetic drift, allows rapid phenotypic changes within their populations. Important changes, such as loss of sensitivity to an antibiotic, can occur over broad

geographic areas in just a few years. Think how many significant metabolic changes could have occurred over even modest time spans in relation to the history of life on Earth. When we introduce the Proteobacteria, the largest group of bacteria, you will see that its different subgroups easily and rapidly adopted and abandoned metabolic pathways under selective pressure from their environments.

The Bacteria

The great majority of known prokaryotes are bacteria. Here we will describe bacterial diversity using a currently popular classification scheme that enjoys considerable support from nucleotide sequence data. More than a dozen monophyletic groups have been proposed under this scheme; we will describe just a few of them here. The higher-order relationships among these groups of prokaryotes are not known. Some biologists describe them as kingdoms, some as subkingdoms, others as phyla. Here we call them groups. We'll pay the closest attention to the Proteobacteria, Cyanobacteria, Spirochetes, Chlamydias, and Firmicutes (see Figure 26.11), but first we mention one property that is shared by members of three other groups.

Some bacteria are heat lovers

Three of the bacterial groups that may have branched out earliest during bacterial evolution are all thermophiles (heat lovers), as are the most ancient of the archaea. This observation supports the hypothesis that the first living organisms were thermophiles that appeared in an environment much hotter than those that predominate today.

The Proteobacteria are a large and diverse group

By far the largest group of bacteria, in terms of number of described species, is the Proteobacteria, sometimes referred to as the purple bacteria. Among the proteobacteria are many species of Gram-negative, bacteriochlorophyll-containing, sulfur-using photoautotrophs. However, this group also includes a dramatically diverse group of bacteria that bear no resemblance to the purple bacteria in phenotype. The mitochondria of eukaryotes were derived from proteobacteria by endosymbiosis.

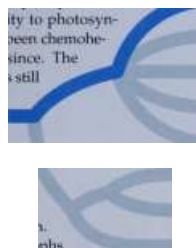
No characteristic demonstrates the diversity of the proteobacteria more clearly than their metabolic pathways (Figure 26.12). The common ancestor of all the proteobacteria was probably a photoautotroph. Early in evolution, two groups of proteobacteria lost their ability to photosynthesize and have been chemoheterotrophs ever since. The other three groups still have photoautotrophic members, but in each group, some evolutionary lines have abandoned photoautotrophy and taken up other modes of nutrition. There are chemoautotrophs and chemoheterotrophs in all

three groups. Why? We can view each of the trends in Figure 26.12 as an evolutionary response to selective pressures encountered as these bacteria encountered new habitats that presented new challenges and opportunities.

Among the proteobacteria are some nitrogen-fixing genera such as *Rhizobium* (see Figure 34.10) and other bacteria that contribute to the global nitrogen and sulfur cycles. *E. coli*, one of the most studied organisms on Earth, is a proteobacterium. So, too, are many of the most famous human pathogens, such as *Yersinia pestis*, *Vibrio cholerae*, and *Salmonella typhimurium*, all mentioned in our discussion of pathogens.

Fungi cause most plant diseases, and viruses cause others, but about 200 plant diseases are of bacterial origin. Crown gall, with its characteristic tumors (Figure 26.13), is one of the most striking. The causal agent of crown gall is *Agrobacterium tumefaciens*, which harbors a plasmid used in recombinant DNA studies as a vehicle for inserting genes into new plant hosts (see Chapter 17).

A change of line color from green to red or blue indicates loss of the ability to photosynthesize.



Cyanobacteria

Spirochetes

Chlamydias

Firmicutes

Crenarchaeota

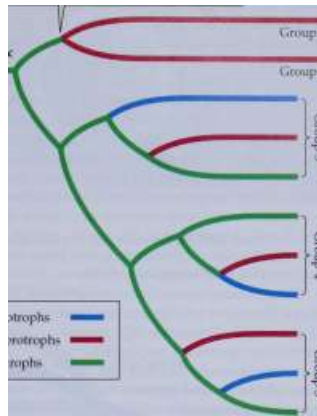
Euryarchaeota

Eukaryotes

Group 1

Photoautotroph ancestor of proteobacteria

Group 2



Chemoautotrophs

Chemoheterotrophs

Photoautotrophs

26.12 The Evolution of Metabolism in the Proteobacteria

The common ancestor of all proteobacteria was probably a photoautotroph. As they encountered new environments, groups 1 and 2 lost the ability to photosynthesize; in the other three groups, some evolutionary lines became chemoautotrophs or chemoheterotrophs.



Crown gall is a plant disease caused by Gram-negative *Agrobacterium tumefaciens*.

26.13 Crown Gall

This colorful tumor is a crown gall growing on the stem of a geranium plant.

470 CHAPTER TWENTY-SIX

The Cyanobacteria are important photoautotrophs

The Cyanobacteria (blue-green bacteria) require only water, nitrogen gas, oxygen, a few mineral elements, light, and carbon dioxide to survive. They use chlorophyll *a* for photosynthesis and liberate oxygen gas; many species also fix nitrogen. Their photosynthesis was the basis of the "oxygen revolution" that transformed Earth's atmosphere.

Cyanobacteria carry out the same type of photosynthesis that is characteristic of eukaryotic photosynthesizers. They contain elaborate and highly organized internal membrane systems called photosynthetic lamellae, or thylakoids (Figure 26.14). The chloroplasts of photosynthetic eukaryotes are derived from an endosymbiotic cyanobacterium.

Cyanobacteria may associate in colonies or live free as single cells. Depending on the species and on growth conditions, colonies of cyanobacteria may range from flat sheets one cell thick to spherical balls of cells.

Some filamentous colonies differentiate into three cell types: vegetative cells, spores, and heterocysts (Figure 26.15). Vegetative cells photosynthesize, spores are resting cells that can eventually develop into new filaments, and heterocysts are cells specialized for nitrogen fixation. All of the known cyanobacteria with heterocysts fix nitrogen. Heterocysts also have a role in reproduction: When filaments break apart to reproduce, the heterocyst may serve as a breaking point.

Thylakoid membranes

Heterocyst

Spore (a "resting" cell)

Vegetative cells

(a) *Anabaena* sp.

2 μm

(b)



A thick wall separates the cytoplasm of the nitrogen-fixing heterocyst from the surrounding environment.

0.6 μm

Proteobacteria



PaeS**?^ *j»- 3k3

0.5 μm

26.14 Thylakoids in Cyanobacteria

This cyanobacterium was prepared by the freeze-etch technique to emphasize the extensive system of internal membranes. These photosynthetic thylakoid membranes are present through most of the cytoplasm and clearly identify the specimen as a cyanobacterium, even though the exact species is not identified here.

26.75 Cyanobacteria

(a) *Anabaena* is a genus of colonial, filamentous cyanobacteria. The vegetative cells are photosynthetic. (b) A thin neck attaches a heterocyst to each of two other cells in a colony, (c) Cyanobacteria appear in enormous numbers in some environments. This California pond has experienced eutrophication: Phosphorus and other nutrients generated by human activity have accumulated in the pond, feeding an immense green mat—commonly referred to as "pond scum"—made up of several species of unicellular cyanobacteria.

Spirochetes look like corkscrews

Spirochetes are Gram-negative bacteria characterized by unique structures called axial filaments, fibrils running through the periplasmic space (see Figure 26.5a). The cell body is a long cylinder coiled into a spiral (Figure 26.16). The axial filaments begin at either end of the cell and overlap in



Treponema pallidum ..

26.76 A Spirochete

This corkscrew-shaped spirochete causes syphilis in humans.

the middle, and there are typical basal rings where they are attached to the cell wall. Many spirochetes live in humans as parasites. Others live free in mud or water.

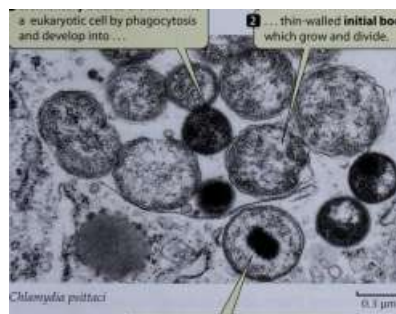
Chlamydias are extremely small

Chlamydias are among the smallest bacteria (0.2-1.5 μm in diameter). They can live only as parasites within the cells of other organisms. These tiny spheres are unique prokaryotes because of their complex life cycle, which involves two different forms of cells (Figure 26.17). In humans, various strains of chlamydias cause eye infections (especially trachoma), sexually transmitted disease, and some forms of pneumonia.

I Elementary bodies are taken into a eukaryotic cell by phagocytosis and develop into...

... thin-walled initial bodies,

which grow and divide.



Chlamydia psittaci

Q Initial bodies reorganize into elementary bodies, which are liberated by the rupture of the host cell.

26.77 Chlamydias Change Form during Their Life Cycle

Elementary bodies and initial bodies are the two major phases of the life cycle of a chlamydia.



Endospore



Clostridium tetani ''

1.2 μm

26.18 The Endospore: A Structure for Waiting Out

Bad Times

This firmicute, which causes tetanus, produces endospores as resistant resting structures.

Most Firmicutes are Gram-positive

The Firmicutes are sometimes referred to as the Gram-positive bacteria, but some firmicutes are Gram-negative, and some have no cell wall at all. Nonetheless, the firmicutes constitute a monophyletic group.

Some firmicutes produce endospores (Figure 26.18)—heat-resistant resting structures—when nutrients become scarce. The bacterium replicates its DNA and encapsulates one copy, along with some of its cytoplasm, in a tough cell wall heavily thickened with peptidoglycan and surrounded by a spore coat. The parent cell then breaks down, releasing the endospore. Endospore production is not a reproductive process; the endospore merely replaces the parent cell. The endospore can survive harsh environmental conditions, such as high or low temperatures or drought, because it is dormant—its normal activity is suspended. Later, if it encounters favorable conditions, the endospore becomes metabolically active and divides, forming new cells like the parent. Some endospores apparently can be reactivated even after more than a thousand years of dormancy.

Members of this endospore-forming group include the many species of *Bacillus* and *Clostridium*. The toxins produced by *C. botulinum* are among the most poisonous ever discovered; the lethal dose for humans is about one-millionth of a gram (1 µg).

The genus *Staphylococcus*—the staphylococci—includes firmicutes that are abundant on the human body surface; they are responsible for boils and

Proteobacteria

Cyanobacteria



Crenarchaeota

Euryarchaeota

Eukaryotes

472 CHAPTER TWENTY-SIX

J!*«t?

:1

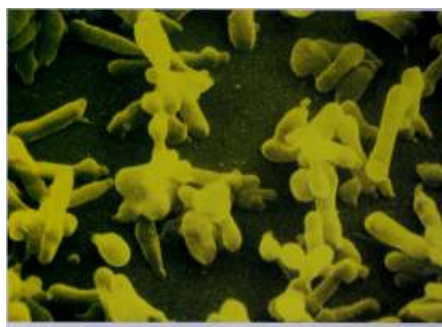
■**«*.,

Staphylococcus aureus

26.19 Gram-Positive Firmicutes

"Grape clusters" are the usual arrangement of Gram-positive staphylococci.

12 |im



Mycoplasma gallisepticum

0.4 µm

26.21 The Tiniest Living Cells

Containing only about one-fifth as much DNA as *E. coli*, mycoplasmas are the smallest known bacteria.

many other skin problems (Figure 26.19). *S. aureus* is the best-known human pathogen; it is found in 20 to 40 percent of normal adults (and in 50 to 70 percent of hospitalized adults). It can cause respiratory, intestinal, and wound infections, in addition to skin diseases.

Actinomycetes are firmicutes that develop an elaborately branched system of filaments (Figure 26.20). These bacteria closely resemble the filamentous bodies of fungi. Some actinomycetes reproduce by forming chains of spores at the tips of the filaments. In the species that do not form spores, the branched, filamentous growth ceases and the structure breaks up into typical cocci or rods, which then reproduce by fission.

The actinomycetes include several medically important bacteria. *Mycobacterium tuberculosis* causes tuberculosis. *Streptomyces* produces streptomycin, as well as hundreds of



Actinomyt

10 urn

26.20 Filaments of an Actinomycete

These branching filaments are visualized with a fluorescent stain. This species is part of the normal flora in the human tonsils, mouth, intestinal tract, and lungs, but will invade body tissues and cause severe abscesses when afforded the opportunity.

other antibiotics, including several dozen in general use. We derive most of our antibiotics from members of the actinomycetes.

Another interesting group of firmicutes, the mycoplasmas, lack cell walls, although some have a stiffening material outside the plasma membrane. Some of them are the smallest cellular creatures ever discovered—they are even smaller than chlamydias (Figure 26.21). The smallest mycoplasmas capable of growth have a diameter of about 0.2 μm , and they are small in another crucial sense: They have less than half as much DNA as do most other prokaryotes. It has been speculated that the amount of DNA in a mycoplasma may be the minimum amount required to code for the essential properties of a living cell.

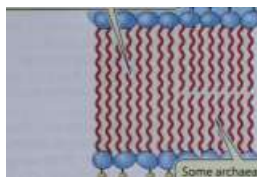
The Archaea

The domain Archaea consists mainly of prokaryotic genera that live in habitats notable for characteristics such as extreme salinity (salt content), low oxygen concentration, high temperature, or high or low pH. On the face of it, the Archaea do not seem to belong together as a group; in fact, some evidence suggests that the domain Archaea is para-phyletic. One current classification scheme treats the domain as two kingdoms: Euryarchaeota and Crenarchaeota. In fact, we know very little about the phylogeny of archaea, in part because the study of archaea is still in its early stages. We do know that archaea share certain characteristics.

The Archaea share some unique characteristics

Two characteristics shared by all archaea are the absence of peptidoglycan in their cell walls and the presence of lipids of distinctive composition in their cell membranes (see Table 26.1). The base sequences of their ribosomal RNA's support a close evolutionary relationship among them. Their separation from the Bacteria and Eukarya was clari-

Long-chain hydrocarbons with glycerol at both ends span the membrane of some archaea.



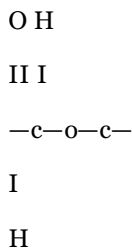
Some archaean hydrocarbons fit the same membrane template as do the fatty acids of bacteria and eukaryotes.

26.22 Membrane Architecture in Archaea

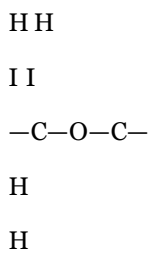
The long-chain hydrocarbons of archaean membranes are branched, and may have glycerol at both ends. This structure still fits into a biological membrane, however; in fact, all three domains have similar membrane structures.

fied when biologists sequenced the first archaean genome: It consisted of 1,738 genes, more than half of which were unlike any genes ever found in the other two domains.

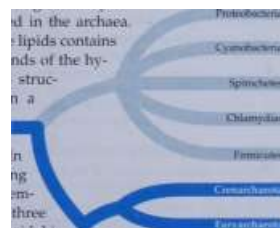
The unusual lipids in the membranes of archaea deserve some description. They are found in all archaea, and in no bacteria or eukaryotes. Most membrane lipids of bacteria and eukaryotes contain unbranched long-chain fatty acids connected to glycerol by ester linkages:



(see also Figure 3.19). In contrast, archaean membrane lipids contain long-chain hydrocarbons connected to glycerol by ether linkages:



In addition, the long-chain hydrocarbons are branched in the archaea. One class of these lipids contains glycerol at both ends of the hydrocarbons. This structure still fits in a biological membrane, as shown in Figure 26.22. In spite of the striking difference in membrane lipids, all three domains have lipid bi-layer membranes with similar overall structures, dimensions, and functions.



Most Crenarchaeota live in hot, acidic places

Most known Crenarchaeota are both thermophilic (heat-loving) and acidophilic (acid-loving). Members of the genus *Sulfolobus* live in hot sulfur springs at temperatures of 70-75°C. They die of "cold" at 55°C (131°F). Hot sulfur springs are also extremely acidic. *Sulfolobus* grows best in the pH range from 2 to 3, but it readily tolerates pH values as low as 0.9. Some acidophilic hyperthermophiles maintain an internal pH near 7 (neutral) in spite of the acidity of their environment. These and other hyperthermophiles thus thrive where very few other organisms can even survive (Figure 26.23).

The Euryarchaeota live in many amazing places

Some species of Euryarchaeota, once assigned to unrelated bacterial groups, share the property of producing methane (CH₄) by reducing carbon dioxide. All of these methane producers are obligate anaerobes, and methane production is the key step in their energy metabolism. Comparison of rRNA nucleotide sequences revealed a close evolutionary relationship among all these methanogens.

Methanogens release approximately 2 billion tons of methane gas into Earth's atmosphere each year, accounting for all the methane in our air, including that associated with mammalian belching. Approximately a third of this methane comes from methanogens in the guts of grazing herbivores such as cows.

26.23 Some Would Call It Hell; Archaea Call It Home

Masses of heat- and acid-loving archaea form an orange mat inside a volcanic vent on the island of Kyushu, Japan. Sulfurous residue is visible at the edges of the archaean mat.





26.24 Extreme Halophiles

Commercial seawater evaporating ponds, such as these in San Francisco Bay, are attractive homes for salt-loving archaea.

One methanogen, *Methanopyrus*, lives on the ocean bottom near blazing volcanic vents. *Methanopyrus* can survive and grow at 110°C. It grows best at 98°C and not at all at temperatures below 84°C.

Another group of Euryarchaeota, the extreme halophiles (salt lovers), lives exclusively in very salty environments. Because they contain pink carotenoids, they can be seen easily under some circumstances (Figure 26.24). Halophiles grow in the Dead Sea and in brines of all types: Pickled fish may sometimes show reddish pink spots that are colonies of halophilic archaea. Few other organisms can live in the saltiest of the homes that the strict halophiles occupy; most would "dry" to death, losing too much water to the hypertonic environment. Strict halophiles have been found in lakes with pH values as high as 11.5—the most alkaline environment inhabited by living organisms, almost as alkaline as household ammonia.

Some of the extreme halophiles have a unique system for trapping light energy and using it to form ATP—without using any form of chlorophyll—when oxygen is in short supply. They use the pigment retinal (also found in the vertebrate eye) combined with a protein to form bacteriorhodopsin, and form ATP by a chemiosmotic mechanism of the sort described in Figure 7.12.

Another member of the Euryarchaeota, *Thermoplasma*, has no cell wall. It is thermophilic and acidophilic, its metabolism is aerobic, and it lives in coal deposits. It has the smallest genome among the archaea, and perhaps the smallest (along with the mycoplasmas) of any free-living organisms—1,100,000 base pairs.

This chapter has provided a brief summary of two of the e domains of the living world. The world of the eukaryotes, both unicellular and multicellular, will be the subject of the next seven chapters.

Chapter Summary

Why Three Domains?

- Living organisms can be divided into three domains: Bacteria, Archaea, and Eukarya. Both the Archaea and the Bacteria are prokaryotic, but they differ from each other more radically than do the Archaea from the Eukarya, which constitute the rest of the living world.
- The evolutionary relationships of the three domains were first revealed by their rRNA sequences. The common ancestor of all three domains lived more than 3 billion years ago, and the common ancestor of the Archaea and Eukarya at least 2 billion years ago. Review Figure 26.2 and Table 26.1

General Biology of the Prokaryotes

- The prokaryotes are the most numerous organisms on Earth, and they occupy an enormous variety of habitats.
- Most prokaryotes are cocci, bacilli, or spiral forms. Some link together to form associations, but very few are truly multicellular. Review Figure 26.3
- Prokaryotes lack nuclei, membrane-enclosed organelles, and cytoskeletons. Their chromosomes are circular. They often contain plasmids. Some prokaryotes contain internal membrane systems. Review Figure 26.4
- Many prokaryotes move by means of flagella, gas vesicles, or gliding mechanisms. Prokaryotic flagella rotate rather than beat.
- Prokaryotic cell walls differ from those of eukaryotes. Bacterial cell walls generally contain peptidoglycan. Differences in peptidoglycan content result in different reactions to the Gram stain. Review Figure 26.7
- Prokaryotes reproduce asexually by fission, but also exchange genetic information.

► Prokaryotes have diverse metabolic pathways and nutritional modes. They include obligate anaerobes, facultative anaerobes, and obligate aerobes. The major nutritional types are photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs. Some prokaryotes base their energy metabolism on nitrogen- or sulfur-containing ions. Review Figure 26.8 and Table 26.2

Prokaryotes in Their Environments

► Some prokaryotes play key roles in global nitrogen and sulfur cycles. Important players in the nitrogen cycle are the nitrogen fixers, nitrifiers, and denitrifiers. Review Figure 26.10

► Photosynthesis by cyanobacteria generated the oxygen gas that permitted the evolution of aerobic respiration and the appearance of present-day eukaryotes.

► Many prokaryotes live in or on other organisms, with neutral, beneficial, or harmful effects.

► A small minority of bacteria are pathogens. Pathogens vary with respect to their invasiveness and toxigenicity. Some produce endotoxins, which are rarely fatal; others produce exotoxins, which tend to be highly toxic.

Prokaryote Phylogeny and Diversity

► Phylogenetic classification of prokaryotes is now based on rRNA sequences and other molecular evidence.

► Lateral gene transfer among prokaryotes, which has occurred throughout evolutionary history, makes it difficult to infer prokaryote phylogeny.

► Evolution, powered by mutation, natural selection, and genetic drift, can proceed rapidly in prokaryotes because they are haploid and can multiply rapidly.

BACTERIA AND ARCHAEA: THE PROKARYOTIC DOMAINS 475

The Bacteria

► There are far more known bacteria than known archaea. One phylogenetic classification of the domain Bacteria groups them into more than a dozen groups.

► The most ancient bacteria, like the most ancient archaea, may be thermophiles, suggesting that life originated in a hot environment.

► All four nutritional types occur in the largest bacterial group, the Proteobacteria. Metabolism in different groups of proteobacteria has evolved along different lines. Review Figure 26.12

► Cyanobacteria, unlike other bacteria, photosynthesize using the same pathways plants use. Many cyanobacteria fix nitrogen.

► Spirochetes move by means of axial filaments.

► Chlamydias are tiny parasites that live within the cells of other organisms.

► Firmicutes are diverse; some of them produce endospores as resting structures that resist harsh conditions. Actinomycetes, some of which produce important antibiotics, grow as branching filaments.

► Mycoplasmas, the tiniest living things, lack conventional cell walls. They have very small genomes.

The Archaea

► Archaea have cell walls lacking peptidoglycan, and their membrane lipids differ from those of bacteria and eukaryotes, containing branched long-chain hydrocarbons connected to glycerol by ether linkages. Review Figure 26.22

► The domain Archaea can be divided into two kingdoms: Crenarchaeota and Euryarchaeota.

► Crenarchaeota are heat-loving and often acid-loving archaea.

► Methanogens produce methane by reducing carbon dioxide. Some methanogens live in the guts of herbivorous animals; others occupy high-temperature environments on the ocean floor.

► Extreme halophiles are salt lovers that often lend a pinkish color to salty environments; some halophiles also grow in extremely alkaline environments.

► Archaea of the genus *Thermoplasma* lack cell walls, are thermophilic and acidophilic, and have a tiny genome (1,100,000 base pairs).

For Discussion

1. Why do systematic biologists find rRNA sequence data more useful than data on metabolism or cell structure for classifying prokaryotes?

2. Why does lateral gene transfer make it so difficult to arrive at agreement on phylogeny?
3. Differentiate among the members of the following sets of related terms:
 - a. prokaryotic/eukaryotic
 - b. obligate anaerobe/facultative anaerobe/obligate aerobe
 - c. photoautotroph/photoheterotroph/chemoautotroph/ chemoheterotroph
 - d. Gram-positive/Gram-negative
4. Why are the endospores of firmicutes not considered to be reproductive structures?
5. Until fairly recently, the cyanobacteria were called blue-green algae and were not grouped with the bacteria. Suggest several reasons for this (abandoned) tendency to separate the bacteria and cyanobacteria. Why are the cyanobacteria now grouped with the other bacteria?
6. The actinomycetes are of great commercial interest. Why?
7. Hyperthermophiles are of great interest to molecular biologists and biochemists. Why? What practical concerns might motivate that interest?

21

Protists and the Dawn of the Eukarya



The Bacteria and the Archaea had the living world to themselves for more than a billion years. As we saw in Chapter 26, members of these two domains differ sharply in several important ways—but neither of these prokaryotes is like the single-celled organism shown here. What strikes you the most about this amoeba? Probably the most obvious visible difference between it and the prokaryotes is that the amoeba has numerous compartments—membrane-enclosed organelles.

What are compartments useful for? For one thing, they keep items separate—like keeping greasy tools away from clean socks by storing them in different cabinets. Compartments also keep things together when that makes sense, such as keeping all your socks in one drawer, or keeping all the files for your term paper in a single directory on your computer. Rooms are another example of compartments, and they can be specialized for different activities: One room in the Biological Sciences Building might be set up as a laboratory, while another room serves as a lecture hall, and still another contains special protective seals that prevent radiation or pathogens from escaping.

The single-celled amoeba is an example of an organism that compartmentalizes: It has a cytoskeleton, a nucleus enclosed by a nuclear envelope, and several kinds of organelles. Amoebas are members of the domain Eukarya, and they differ from members of the two prokaryotic domains in other important ways as well.

The flexibility and options that arose once the eukaryotic cell had evolved resulted in a profusion of body forms and myriad specialized functions. Eukaryotic evolution has produced great diversity, especially among the multicellular lineages, but even among the unicellular protists. In both multicellular and unicellular forms, however, there are also many cases of convergent evolution; for example, organisms with an amoeba-like body form arose several times.

Protists Defined

Many modern members of the Eukarya are familiar to us—trees, dogs, and mushrooms, not to mention our-

An Amoeba

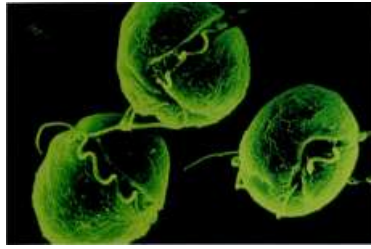
is have several kinds of organelles (seen here as bubble-like compartments). Their flowing pseudopods are constantly changing shape as the amoeba moves and feeds.

selves. These members of the kingdoms Plantae, Animalia, and Fungi are not strange to us. However, amoebas and a dazzling assortment of other eukaryotes, mostly microscopic organisms, don't fit into these three kingdoms. We call all those eukaryotes that are neither plants, animals, nor fungi protists (Figure 27.1; Table 27.1). The protists are not a monophyletic group. Some protists are more closely related to the animals than they are to other protists. Some protists are motile, while others are stationary; some are photosynthetic, while others are heterotrophic; most are unicellular, while some giant kelps are not only multicellular but also huge, sometimes achieving lengths greater than that of a football field.

The origin of the eukaryotic cell was one of the pivotal events in evolutionary history. In this chapter on the protists we describe and celebrate the origin and early diversification of the eukaryotes and the complexity achieved by some single cells. We'll explore some of the diversity of protist body forms, and we'll try to give a sense of developing current views of the evolutionary relationships of some of the protists.

The Origin of the Eukaryotic Cell

The eukaryotic cell differs in many ways from prokaryotic cells. How did it originate? Given the nature of evolutionary processes, the differences cannot all have arisen simultaneously. We think we can make some reasonable guesses



(a) *Gonyaulax* sp.

27.1 Three Eukaryote Protists

(a) Dinoflagellates are photosynthetic unicellular protists. (b) *Giardia* is a unicellular parasite of humans, (c) Giant kelps are some of the world's longest organisms.



(b) *Giardia* sp.

(c) *Macrocystis* sp.

about the important events, bearing in mind that the global environment underwent an enormous change—from anaerobic to aerobic—during the course of these events. As you read this chapter, keep in mind that the steps we sug-

gest are just that: guesses. This version of the story is one of a few under current consideration. We present it as a framework for thinking about this challenging problem, not as a set of facts.

i/.1 Major Monophyletic Protist Groups

GROUP

COMMON NAME

ATTRIBUTES

EXAMPLES

Euglenozoa

Euglenoids Kinetoplastids

Alveolata

Pyrrophyta

Apicomplexa

Ciliophora

Stramenopila

Bacillariophyta

Phaeophyta Oomycota

Rhodophyta Chlorophyta Choanoflagellida

Dinoflagellates Ciliates

Diatoms

Brown algae Water molds, powdery mildews

Red algae

Green algae"

Unicellular, with flagella Mostly photoautotrophic Have a single, large mitochondrion

Unicellular; cavities (alveoli)

below cell surface Pigments give golden-brown color Apical complex for penetration of host Cilia; two types of nuclei

Two unequal flagella, one with hairs Unicellular; photoautotrophic;

two-part walls Multicellular; marine; photoautotrophic Mostly coenocytic; heterotrophic

No flagella; photoautotrophic; phycocyanin

Photoautotrophic

Resemble sponge cells; heterotrophic

Euglena Trypanosoma

Gonyaulax

Plasmodium Paramecium

Fucus, Macrocystis Saprolegnia

Chondrus

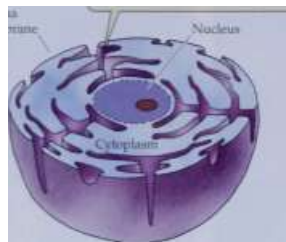
Chlamydomonas, Ulothrix

"The green algae do not constitute a monophyletic group. The Chlorophyta are one lineage of green algae that qualifies as a monophyletic group; another lineage gave rise to the plant kingdom.

478 CHAPTER TWENTY-SEVEN

Plasma membrane

Infolding of the plasma membrane adds surface area without increasing the cell's volume.



27.2 Membrane Infolding

The loss of the rigid prokaryotic cell wall allowed the plasma membrane to elaborate inward and create more surface area.

The modern eukaryotic cell arose in several steps

The essential steps in the origin of the eukaryotic cell include

- ▶ The origin of a flexible cell surface
- ▶ The origin of a cytoskeleton
- ▶ The origin of a nuclear envelope
- ▶ The appearance of digestive vesicles
- ▶ The endosymbiotic acquisition of certain organelles

what a flexible cell surface allows. Many ancient fossil prokaryotes look like rods, and we presume that they, like most present-day prokaryotic cells, had firm, cell walls. The first step toward the eukaryotic condition may have been the loss of the cell wall by an ancestral prokaryotic cell. This may not seem like an obvious first step, but consider the possibilities open to a flexible cell without a wall.

First, think of cell size. As a cell grows, its surface area-to-volume ratio decreases (see Figure 4.2). Unless the surface is flexible and can fold inward and elaborate itself, creating more surface area for gas and nutrient exchange (Figure 27.2), the cell volume will reach an upper limit. With a surface flexible enough to allow infolding, the cell can exchange materials with its environment rapidly enough to sustain a larger volume and more rapid metabolism. Further, a flexible surface can pinch off bits of the environment, bringing them into the cell by endocytosis (Figure 27.3).

o recall that the chromosome of a prokaryotic

is attached to a site on its plasma membrane (see

Figure 26.4). If that region of the plasma membrane

ne to fold into the cell, the first step would be taken

Ribosomes-Protective cell wall DNA-

Prokaryotic cell

t

Loss of the cell wall was probably the first step

Infolding increased the surface area for the absorption of nutrients from the surrounding food supply.

Internal membranes studded with ribosomes formed, some of which surrounded the DNA.

f

Cytoskeleton (actin and microtubules) formed.

I As DNA attached to the membrane of an infolded vesicle a precursor of a nucleus formed.

f

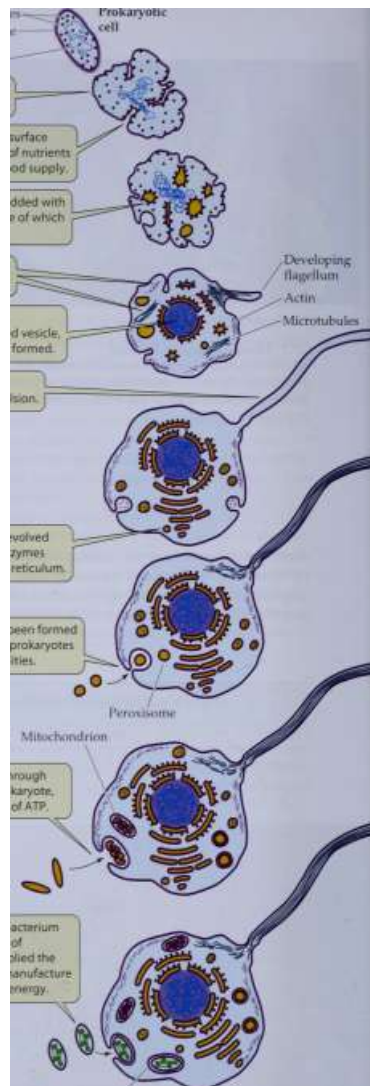
A eukaryotic flagellum formed, enabling propulsion

I Early digestive vesicles evolved into lysosomes using enzymes from early endoplasmic reticulum.

I Peroxisomes may have been formed through endocytosis of prokaryotes with detoxifying capabilities.

| Mitochondria, formed through the endocytosis of a prokaryote enabled the generation of ATP

IE Endocytosis of a cyanobacterium led to the development of chloroplasts, which supplied the cell with the means to manufacture materials utilizing solar energy.



Chloroplast

27.3 From Prokaryotic Cell to Eukaryotic Cell

One possible evolutionary sequence is shown here. The exact sequence, of course, is not known.

PROTISTS AND THE DAWN OF THE EUKARYA 479

toward the evolution of a nucleus, the key feature of the eukaryotic cell.

CHANGES IN CELL STRUCTURE AND FUNCTION. Early Steps in

the evolution of the eukaryotic cell are likely to have included three advances: the formation of ribosome-studded internal membranes, some of which surrounded the DNA (see Figure 27.3); the appearance of a cytoskeleton and the evolution of digestive vesicles.

A cytoskeleton made up of actin fibers and microtubules would allow the cell to manage changes in shape, to distribute daughter chromosomes, and to move materials from one part of the now much larger cell to other parts. The origin of the cytoskeleton remains a mystery, heightened by the fact that the genes that encode the cytoskeleton are present in neither bacteria nor archaea. An intriguing and controversial suggestion is that a fourth domain of life, now long extinct, originated these genes and transferred them laterally to an ancestor of the early eukaryotes.

From an intermediate kind of cell, the next advance was probably to a cell that we could call a phagocyte—a motile cell that could prey on other cells by engulfing and digesting them. The first true eukaryote possessed a cytoskeleton and a nuclear envelope. It may have had an associated endoplasmic reticulum and Golgi apparatus, and perhaps one or more flagella of the eukaryotic type. Notice how much of the progress to this point was made possible by the loss of the cell wall and the elaboration of what was originally the plasma membrane.

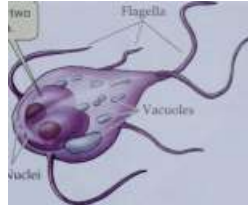
endosymbiosis and organelles. While the processes already outlined were taking place, the cyanobacteria were very busy, generating oxygen gas as a product of photosynthesis. The increasing O_2 levels in the atmosphere had disastrous consequences for most other living things, because most living things of the time (archaea and bacteria) were unable to tolerate the newly aerobic, oxidizing environment. But some prokaryotes managed to cope, and—fortunately for us—so did some of the ancient phagocytes.

According to one hypothesis, the key to the survival of early phagocytes was the ingestion and incorporation of a prokaryote

that became symbiotic within the phagocyte and evolved into the peroxisomes of today (see Figure 27.3). These organelles were able to disarm the toxic products of oxygen action, such as hydrogen peroxide. This association may have been the first important endosymbiosis in the evolution of the eukaryotic cell.

In Chapter 4 we introduced the concept of endosymbiosis (organisms living together, one inside the other). A crucial endosymbiotic event in the history of the Eukarya was the incorporation of a proteobacterium that evolved into the mitochondrion. Upon completion of this step, the basic modern eukaryotic cell was complete. Some very important eukaryotes are the result of yet another endosymbiotic step, the incorporation of prokaryotes related to today's cyanobacteria, which became chloroplasts. We'll see how this happened later in the chapter.

This unicellular *Giardia* has two nuclei but no mitochondria.



Nuclei

27.4 *Giardia*: A Protist without Mitochondria

Current evidence indicates that *Giardia* is descended from an ancestor that possessed mitochondria.

"Archaezoa": The little kingdom that was

The hypothesis that the eukaryotic nucleus evolved before the mitochondrion gained early support from the existence of a few unicellular eukaryotes, such as *Giardia*, that lack mitochondria. *Giardia lamblia* is a familiar parasite that contaminates water supplies and causes the intestinal disease giardiasis (Figure 27.4). This tiny organism has no mitochondria, chloroplasts, or other membrane-enclosed organelles, but it contains two nuclei bounded by nuclear envelopes, and it has a cytoskeleton. Some biologists treated such eukaryotes without mitochondria as the modern descendants of a hypothetical ancient group, which they called "archaezoans." It was later learned that at least some archaezoans may have descended from eukaryotes that lost their mitochondria. Research sometimes takes surprising twists and turns! We no longer speak of a kingdom of archaezoans. However, the existence of such organisms today shows that eukaryotic life is feasible without mitochondria, and the eukaryotes that lack mitochondria are the focus of much attention.

Many uncertainties remain

Several uncertainties cloud our current understanding of the origins of eukaryotic cells. Lateral gene transfer complicates the study of eukaryotic origins just as it complicates the study of relationships among the prokaryote lineages. At the same time, it may not have been extensive enough to account for the fact that, as genetic studies advance, more and more genes of bacterial origin are being found in eukaryotes.

An endosymbiotic origin of mitochondria and chloroplasts accounts for the presence of bacterial genes encoding enzymes for energy metabolism (respiration and photosynthesis), but it does not explain the presence of many other bacterial genes. The eukaryotic genome clearly is a mixture of genes with two distinct origins. A recent suggestion is that the Eukarya might have arisen from the mutualistic fusion (not endosymbiosis) of a Gram-negative bacterium and an archaean. There are many interesting ideas about eukaryotic origins awaiting additional data and analysis.

480 CHAPTER TWENTY-SEVEN

We can expect that these and other questions will yield to additional research. Let's leave our speculations about the origin of the protists for the moment and examine what we do know about them.

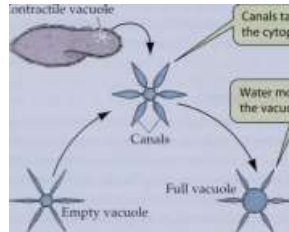
General Biology of the Protists

Most protists are aquatic. Some live in marine environments, others in fresh water, and still others in the body fluids of other organisms. The slime molds inhabit damp soil and the moist, decaying bark of rotting trees. Many other protists also live in soil water, some of them contributing to the global nitrogen cycle by preying on soil bacteria and recycling their nitrogen compounds to nitrates.

Protists are strikingly diverse in their structure, but not so diverse in their metabolism as the prokaryotes—in fact, some of the eukaryotes' most important metabolic pathways were "borrowed" from bacteria through endosymbiosis. However, protists do display a number of nutritional modes. Some are autotrophs, some are heterotrophs, and some switch with ease between the autotrophic and heterotrophic modes of nutrition.

Some protists, formerly classified as animals, are sometimes referred to as protozoans, although biologists increasingly regard this term as inappropriate because it lumps together protist groups that are phylogenetically distant from one another. Most protozoans are ingestive heterotrophs. There are several kinds of photosynthetic protists that some biologists still refer to as algae (singular alga). Although these two terms—protozoans and algae—are useful in some contexts, they do not correspond with natural phy-

Contractile vacuole



Canals take up water from the cytoplasm.

Water moves from canals to the vacuole.

//\ Empty vacuole



The pore opens and the vacuole contracts. Contraction of the vacuole expels water from the cell.

27.5 Contractile Vacuoles Ball Out Excess Water

Water constantly enters freshwater protists by osmosis. A pore in the cell surface allows the contractile vacuole to expel the water it accumulates.

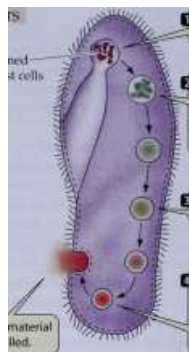
EXPERIMENT

Question: Where—and how—does *Paramecium* digest its food? METHOD Yeast cells are stained with Congo red, a pH indicator.

RESULTS

Stained yeast cells

f



A food vacuole forms around yeast cells.

The change in color shows that the vacuole has become acidic, like your stomach; acid helps digest the yeast cells.

Digestion continues.

Waste material is expelled.

As products of digestion move into the cytosol, the pH increases in the vacuole. The dye becomes red again.

Conclusion: Digestion, assisted by low pH, took place in the food vacuole.

27.6 Food Vacuoles Handle Digestion and Excretion

An experiment with *Paramecium* demonstrates the function of food vacuoles. *Paramecium* ingests food by way of the oral groove at the left. The dye Congo red turns green at acidic pH and red at neutral or basic pH.

logeny, and we generally avoid them in this book except as parts of descriptive names such as "brown algae."

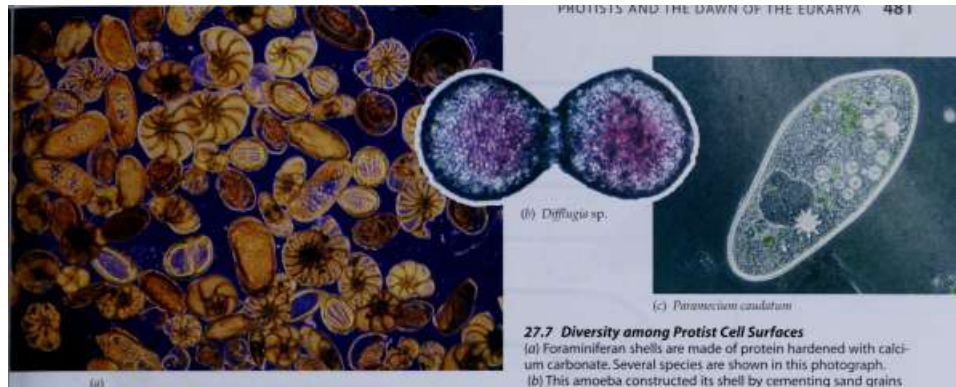
Protists have diverse means of locomotion

Although a few protist groups consist entirely of nonmotile organisms, most groups include cells that move, either by amoeboid motion, by ciliary action, or by means of flagella. In amoeboid motion, the cell forms pseudopods ("false feet") that

are extensions of its constantly changing body mass. Cells such as the amoeba on page 476 simply extend a pseudopod and then flow into it. Cilia are tiny, hairlike organelles that beat in a coordinated fashion to move the cell forward or backward (see Figure 4.24). A eukaryotic flagellum moves like a whip; some flagella push the cell forward, others pull the cell forward.

Vesicles perform a variety of functions

Unicellular organisms tend to be of microscopic size. As we noted above, an important reason that cells are small is that they need enough membrane surface area in relation to their volume to support the exchange of materials required for their existence. Many relatively large unicellular protists minimize this problem by having membrane-enclosed vesicles of various types that increase their effective surface area. As we saw in Chapter 5, organisms living in fresh water are hypertonic to their environments. Many freshwater pro-



tists address this problem by means of vesicles that contract to excrete excess water. Members of several protist groups have such contractile vacuoles. Because these organisms have a higher concentration of solutes than their freshwater environment does, they constantly take in water by osmosis. The excess water collects in the contractile vacuole and is then pushed out (Figure 27.5).

It is easy to confirm that bailing out water is the principal function of the contractile vacuole. First, we can observe some protists under a light microscope and note the rate at which the vacuoles are contracting—they look like little eyes winking. Then we can place other protists of the same species in solutions of differing osmotic potential. The less negative the osmotic potential of the surrounding solution, the more hypertonic the cells are, and the faster water rushes into them, causing the contractile vacuoles to pump more rapidly. Conversely, the contractile vacuoles will stop pumping if the solute concentration of the medium is increased so that it is equal to that of the cells.

A second important type of vesicle found in many protists is the food vacuole. Protists such as Paramecium engulf solid food by endocytosis, forming a food vacuole within which the food is digested (Figure 27.6). Smaller vesicles containing digested food pinch away from the food vesicle and enter the cytoplasm. These tiny vesicles provide a large surface area across which the products of digestion may be absorbed by the rest of the cell.

The cell surfaces of protists are diverse

A few protists, such as some amoebas, are surrounded by only a plasma membrane, but most have stiffer surfaces that maintain the structural integrity of the cell. Many protists have cell walls, which are often complex in structure. Other protists that lack cell walls have a variety of ways of strengthening their surfaces. Some have internal "shells," which the organism either produces itself, as foraminiferans do, or makes from bits of sand and thickenings imme-

27.7 Diversity among Protist Cell Surfaces

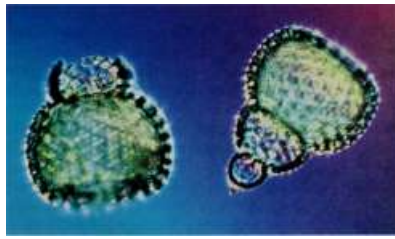
(a) Foraminiferan shells are made of protein hardened with calcium carbonate. Several species are shown in this photograph.

(b) This amoeba constructed its shell by cementing sand grains together, (c) Spirals of protein make this Paramecium's surface—known as its pellicle—flexible but resilient.

diately beneath the plasma membrane, as some amoebas do (Figure 27.7).

Many protists contain endosymbionts

Endosymbiosis is very common among the protists, and in some instances both the host and the endosymbiont are protists. Many radiolarians, for example, harbor photosynthetic protists (Figure 27.8). As a result, these radiolarians appear greenish or yellowish, depending on the type of endosymbiont they contain. This arrangement is beneficial to the radiolarian, for it can make use of the food produced by its photosynthetic guest. The guest, in turn, may make use of metabolites made by the host, or it may simply receive physical protection. In other cases, the guest may be a victim, exploited for its photosynthetic products while receiving no benefit itself.

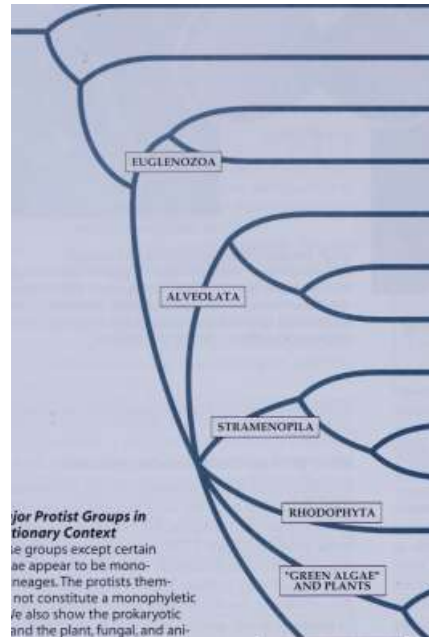


27.8 Protists within Protists

Photosynthetic organisms living as endosymbionts within these radiolarians provide food for the radiolarians, as well as part of the pigmentation seen through their glassy skeletons. Both the endosymbionts and the radiolarians are protists.

482 CHAPTER TWENTY-SEVEN

Common ancestor of all organisms



BACTERIA

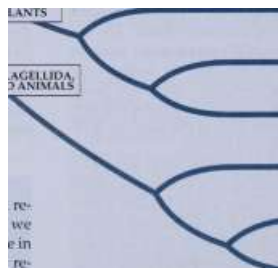
ARCHAEA

27.9 Major Protist Groups in an Evolutionary Context

All of these groups except certain green algae appear to be monophyletic lineages. The protists themselves do not constitute a monophyletic lineage. We also show the prokaryotic domains and the plant, fungal, and animal kingdoms to provide context.

"GREEN ALGAE" AND PLANTS

CHOANOFAGELLATA, FUNGI, AND ANIMALS



Other green algae

Both asexual and sexual reproduction occur among the protists

Although most protists practice both asexual and sexual reproduction, some groups lack sexual reproduction. As we will see, some asexually reproducing protists also engage in genetic recombination, even though it does not directly result in reproduction.

Asexual reproductive processes in the protists include binary fission (simple splitting of the cell, with mitosis followed by cytokinesis), multiple fission (splitting into more than two cells), budding (the outgrowth of a new cell from the surface of an

old one), and the formation of spores (cells that are capable of developing into new organisms). Sexual reproduction also takes various forms. In some protists, as in animals, the gametes are the only haploid cells. In some other protists, by contrast, both diploid and haploid cells undergo mitosis, giving rise to alternation of generations, which will be described later in the chapter.



Euglenoids

Kinetoplastids

Ciliates

Dinoflagellates

Apicomplexans

Water molds



Diatoms

Brown algae



Red algae

Chlorophyta



PLANTS



FUNGI H<



ANIMALS

Choanoflagellates



The diversity of form, habitat, metabolism, locomotion, reproduction, and life cycles found among the protists reflects the diversity of avenues pursued during the early evolution of eukaryotes. Many of these avenues led to great success, judging from the abundance and diversity of today's protists and other eukaryotes.

Protist Diversity

As we have seen, the phylogeny of protists is an area of exciting, challenging research. The marvelous diversity of protist body forms and metabolic lifestyles seems reason enough for a fascination with these organisms, but questions about how the multicellular eukaryotic kingdoms originated from the protists stimulate further interest. Fortunately, the tools of molecular biology, such as rRNA sequencing, make it possible to explore evolutionary relationships among the protists in greater detail and with somewhat greater confidence than previously (see Chapters 24 and 26).

We will discuss several apparently monophyletic groups of protists, as well as a few other groups of more uncertain phylogenetic status. Some biologists refer to many of these monophyletic groups as kingdoms; others refer to them as subkingdoms; still others refer to them as phyla. This choice of words is not of immediate concern to us here, so we'll just call them "groups." We'll describe the Euglenozoa, Alveolata, Stramenopila, Rhodophyta, Chlorophyta, and Choanoflagellida (Figure 27.9).

As we shall see, some of the monophyletic protist groups consist of organisms with very diverse body plans. On the other

hand, certain body plans, such as those of amoebas and those of slime molds, have arisen again and again during evolution, in groups only distantly related to one another.

Euglenozoa

The Euglenozoa are a monophyletic group of flagellates: unicellular organisms with flagella. They reproduce asexually by binary fission. There are two subgroups of Euglenozoa: euglenoids and kinetoplastids.

Euglenoids have anterior flagella

The euglenoids possess flagella arising from a pocket at the anterior end of the cell. Euglenoids used to be claimed by the zoologists as animals and by the botanists as plants. They are unicellular flagellates, but many members of the group are photosynthetic.

Figure 27.10 depicts a cell of the genus *Euglena*. Like most other euglenoids, this common freshwater organism has a complex cell structure. It propels itself through the water with one of its two flagella, which may also serve as an anchor to hold the organism in place. The flagellum provides power by means of a wavy motion that spreads from base to tip. The second flagellum is often rudimentary.

Euglena has very flexible nutritional requirements. Many species are always heterotrophic. Other species are fully autotrophic in sunlight, using chloroplasts to synthe-

Photosynthetic chloroplasts are prominent features in a typical *Euglena* cell.

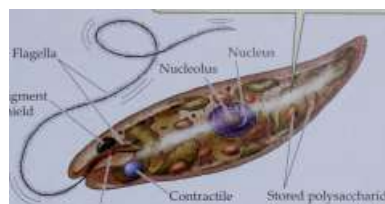


Alveolata

Stramenopila

Rhodophyta

Chlorophyta



Flagella

Pigment shield

Photoreceptor

Contractile vacuole

Stored polysaccharide from photosynthesis

27.10 A Photosynthetic Euglenoid

Several *Euglena* species are among the best-known flagellates. In this species, the second flagellum is rudimentary.

size organic compounds through photosynthesis. The chloroplasts of euglenas are surrounded by three membranes (unlike plant chloroplasts, which have only two). When kept in the dark, these euglenas lose their photosynthetic pigment and begin to feed exclusively on dead organic material floating in the water around them. Such a "bleached" *Euglena* resynthesizes its photosynthetic pigment when it is returned to the light and becomes autotrophic again. But *Euglena* cells treated with certain antibiotics or mutagens lose their photosynthetic pigment completely; neither they nor their descendants are ever autotrophs again. However, those descendants function well as heterotrophs.

Kinetoplastids have mitochondria that edit their own RNA

The kinetoplastids are unicellular, parasitic flagellates with a single, large mitochondrion. That mitochondrion contains a kinetoplast — a unique structure housing DNA and associated proteins. The kinetoplast DNA is of two types, called minicircles and maxicircles. The maxicircles encode enzymes associated with oxidative metabolism, and the mini-circles encode "guides" that accomplish a remarkable type of RNA editing within the mitochondrion.

Some kinetoplastids are human pathogens. Sleeping sickness, one of the most dreaded diseases of Africa, is caused by the parasitic kinetoplastid *Trypanosoma* (Figure 27.11). The vector (intermediate host) for *Trypanosoma* is an insect, the tsetse fly. Carrying its deadly cargo, the tsetse fly bites livestock, wild animals, and even humans, infecting them with the parasite. *Trypanosoma* then multiplies in the mammalian bloodstream and produces toxins. When these parasites invade the nervous

system, the neurological symptoms of sleeping sickness appear and are followed by death. Other trypanosomes cause leishmaniasis, Chagas' disease, and East Coast fever; all are major diseases in the tropics.

484 CHAPTER TWENTY-SEVEN

Undulating membrane of trypanosome



Trypanosoma gambiense

25 (irn

27.11 A Parasitic Kinetoplastid

Trypanosomes, shown here among human red blood cells, cause sleeping sickness in mammals. A flagellum runs along one edge of the cell as part of a structure called the undulating membrane.

Alveolata

The Alveolata are a monophyletic group of unicellular organisms characterized by the possession of cavities called alveoli just below their plasma membranes. They are diverse in body form. The alveolate groups we'll consider here are the dinoflagellates, apicomplexans, and ciliates.

Dinoflagellates are unicellular marine organisms with two flagella

The dinoflagellates are all unicellular, and most are marine organisms. A distinctive mixture of photo-synthetic and accessory pigments gives their chloroplasts a golden-brown color. The dinoflagellates are of great ecological, evolutionary, and morphological interest. They are among the most important primary photosynthetic producers of organic matter in the oceans.

Many dinoflagellates are endosymbionts, living within the cells of other organisms, including various invertebrates and even other marine protists. Dinoflagellates are particularly common endosymbionts in corals, to whose growth they contribute by photosynthesis. Some dinoflagellates are nonphotosynthetic and live as parasites within other marine organisms.

Dinoflagellates have a distinctive appearance (see Figure 17.1a). They have two flagella, one in an equatorial groove around the cell, the other starting at the same point as the first and passing down a longitudinal groove before extending into the surrounding medium.

Euglenozoa

Stramenopila

Rhodophyta

Chlorophyta

Some dinoflagellates reproduce in enormous numbers in warm and somewhat stagnant waters. The result can be a "red tide," so called because of the reddish color of the sea that results from pigments in the dinoflagellates (Figure 27.12). During a red tide, the concentration of dinoflagellates may reach 60 million cells per liter of ocean water. Certain red tide species produce a potent nerve toxin that can kill tons of fish. The genus *Gonyaulax* produces a toxin that can accumulate in shellfish in amounts that, although not fatal to the shellfish, may kill a person who eats the shellfish.

Many dinoflagellates are bioluminescent. In complete darkness, cultures of these organisms emit a faint glow. If you suddenly stir or bubble air through the culture, the organisms each emit numerous bright flashes. A ship passing through a tropical ocean that contains a rich growth of these species produces a bow wave and wake that glow eerily as billions of these dinoflagellates discharge their light systems.

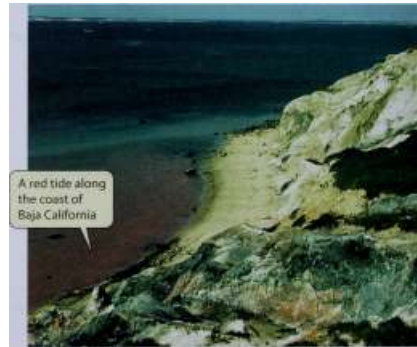
Apicomplexans are parasites with unusual spores

Exclusively parasitic organisms, the apicomplexans derive their name from the apical complex, a mass of organelles contained within the apical end of their spores. These organelles help the apicomplexan spore invade its host tissue. Unlike many other protists, apicomplexans lack contractile vacuoles.

Apicomplexans generally have an amorphous body form like that of an amoeba. This body form has evolved over and over again in parasitic protists. It appears even among parasitic dinoflagellates, a group of organisms whose nonparasitic relatives

have highly distinctive, complex body forms.

Like many obligate parasites, apicomplexans have elaborate life cycles featuring asexual and sexual reproduction



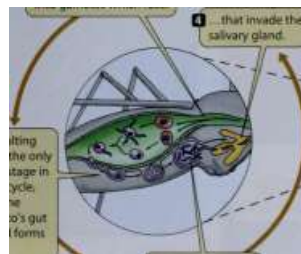
27.12 A Red Tide of Dinoflagellates

By reproducing in astronomical numbers, the dinoflagellate *Gonyaulax tamarensis* can cause a toxic red tide.

EVENTS IN MOSQUITO

EVENTS IN HUMAN

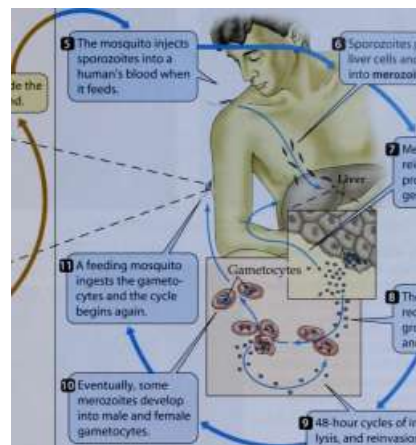
After a mosquito ingests blood, male and female gametocytes develop into gametes which fuse.



Sporozoites penetrate liver cells and develop into merozoites.

The resulting zygote, the only diploid stage in the life cycle, enters the mosquito's gut wall and forms a cyst.

Within the cyst, the zygote gives rise to sporozoites...



Merozoites can reinfect the liver, producing new generations.

A feeding mosquito ingests the gametocytes and the cycle begins again.

They also invade red blood cells, grow and divide, and lyse the cells.

Eventually, some merozoites develop into male and female gametocytes.

27.13 The Life Cycle of an Apicomplexan

Malaria-causing *Plasmodium* species spend part of their life cycle in humans and part in mosquitoes. Sporozoites and merozoites are spores with apical complexes.

by a series of very dissimilar life stages. Often these stages are associated with two different types of host organism.

The best-known apicomplexans are the malarial parasites of the genus *Plasmodium*, a highly specialized group of organisms

that spend part of the life cycle within human red blood cells (Figure 27.13). Although it has been almost eliminated from the United States, malaria continues to be a major problem in many tropical countries. In terms of the number of people infected, malaria is one of the world's most serious diseases.

Female mosquitoes of the genus *Anopheles* transmit *Plasmodium* to humans. The parasite enters the human circulatory system when an infected *Anopheles* mosquito penetrates the human skin in search of blood. The parasites find their way to cells in the liver and the lymphatic system, change their form, multiply, and reenter the bloodstream, attacking red blood cells. The apical complex enables *Plasmodium* to enter human liver cells and red blood cells.

The parasites multiply inside red blood cells, which then burst, releasing new swarms of parasites. If another *Anopheles* bites the victim, the mosquito takes in some of the parasitic *Plasmodium* cells along with blood. The infecting cells develop into gametes, which unite to form zygotes that lodge in the mosquito's gut, divide several times, and

Q 48-hour cycles of invasion, lysis, and reinvasion cause the characteristic fevers and chills of the host victim.

move into its salivary glands, from which they can be passed on to another human host. Thus, *Plasmodium* is an extracellular parasite in the mosquito vector and an intracellular parasite in the human host.

Malaria kills more than a million people each year, and *Plasmodium* has proved to be a singularly difficult pathogen to attack. The *Plasmodium* life cycle is best broken by the removal of stagnant water, in which mosquitoes breed. The use of insecticides to reduce the *Anopheles* population can be effective, but their benefits must be weighed against the possible ecological, economic, and health risks posed by the insecticides themselves. However, there is now new hope in the form of a genome-sequencing project that targets the common form of *Plasmodium*. Scheduled to be completed by the year 2002, this project may provide the information needed to end the epidemic.

Ciliates have two types of nuclei

The ciliates are so named because they characteristically have hairlike cilia. This group is noteworthy for its diversity and ecological importance (Figure 27.14). Almost all ciliates are heterotrophic (a few contain photosynthetic endosymbionts), and they are much more specialized in body form than are most flagellates and other protists.

The definitive characteristic of ciliates is the possession of two types of nuclei, from one to as many as a thousand large macronuclei and, within the same cell, from one to eighty micronuclei. The micronuclei, which are typical eukaryotic nuclei, are essential for genetic recombination. The

486 CHAPTER TWENTY-SEVEN

Cilia

Tentacles



(a) *Paramecium bursaria*

27.14 Diversity among the Ciliates (a) A free-swimming organism, this *Paramecium* belongs to a ciliate group whose members have many cilia of uniform length. (b) Members of this subgroup have cilia on their mouthparts. (c) In this group, tentacles replace cilia as development proceeds. (d) This ciliate "walks" on fused cilia called cirri that project from its body. Other cilia are fused into flat sheets that sweep food particles into the oral cavity.

10 urn

(b) *Epistylis* Cirri

sp.





(c) Paracineteta sp.

20 μ m

(d) Euplotes sp.

25 μ m

macronuclei are derived from micronuclei. Each macronucleus contains many copies of the genetic information, packaged in units containing very few genes each; the macronuclear DNA is transcribed and translated to regulate the life of the cell. Although we do not know how this system of macro- and micronuclei came into being, we do know something about the behavior of these nuclei, which we will discuss after describing the body plan of one important ciliate, Paramecium.

A closer look at one ciliate. Paramecium, a frequently studied ciliate genus, exemplifies the complex structure and behavior of ciliates (Figure 27.15 a). The slipper-shaped cell is covered by an elaborate pellicle, a structure composed principally of an outer membrane and an inner layer of closely packed, membrane-enclosed sacs (the alveoli) that surround the bases of the cilia. Defensive organelles called trichocysts are also present in the pellicle. In response to a threat, a microscopic explosion expels the trichocysts in a few milliseconds, and they emerge as sharp darts, driven forward at the tip of a long, expanding filament (Figure 27.15b).

The cilia provide a form of locomotion that is generally more precise than locomotion by flagella or pseudopods. A

Figure 27.15 Anatomy of Paramecium

(a) The major structures of a typical Paramecium, (b) A trichocyst discharged from beneath the pellicle of a Paramecium has a sharp point and a straight filament.

Paramecium can direct the beat of its cilia to propel itself either forward or backward in a spiraling manner (Figure 27.16). It can also back off swiftly when it encounters a barrier or a negative stimulus. The coordination of ciliary beating is probably the result of a differential distribution of ion channels in the plasma membrane near the two ends of the cell.

REPRODUCTION WITHOUT SEX, AND SEX WITHOUT REPRODUCTION.

Paramecia reproduce asexually by binary fission. The micro-

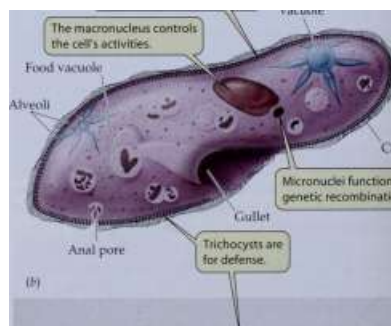
(a)

The complex cell surface structure is called the pellicle.

Contractile vacuole

The macronucleus controls the cell's activities.

Cilia



Micronuclei function in genetic recombination.

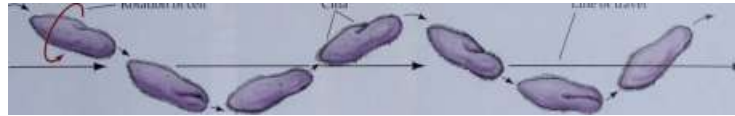




Rotation of cell

Cilia

Line of travel



27.76 "Swimming" with Cilia

Beating its cilia in coordinated waves that progress from one end of the cell to the other, a Paramecium can move in either direction with respect to the long axis of the cell. The cell rotates in a spiral as it travels.

nuclei divide mitotically. The macronuclei divide by a still unknown mechanism following a round of DNA replication.

Paramecia also have an elaborate sexual behavior called conjugation. Two paramecia line up tightly against each other and fuse in the oral region of the body. Nuclear material is extensively reorganized and exchanged over the next several hours (Figure 27.17). As a result of this process, each cell ends up with two haploid micronuclei, one from itself and one from the other cell, which fuse to form a new diploid micronucleus. New macronuclei develop from the micronuclei through a series of dramatic chromosomal rearrangements. The exchange of nuclei is fully reciprocal—each of the two paramecia gives and receives an equal amount of DNA. The two organisms then separate and go their own ways, each equipped with new combinations of alleles.

Conjugation in Paramecium is a sexual process of genetic recombination, but it is not a reproductive process. The same two cells that begin the process are there at the end, and no new cells are created. As a rule, each clone of paramecia must periodically conjugate. Experiments have shown that if some species are not permitted to conjugate, the asexual

27.77 Paramecia Achieve Genetic Recombination by Conjugating

Conjugating Paramecium individuals exchange micronuclei, thereby permitting genetic recombination. After conjugation, the cells separate and continue their lives as two individuals.

clones can live through no more than approximately 350 cell divisions before they die out.

Stramenopila

The stramenopiles include three prominent groups, two of which are photosynthetic. The two flagella of a stramenopile cell are typically unequal in length. The longer of the two bears rows of tubular hairs. Some stramenopiles lack flagella, but they are presumed to be descended from ancestors that possessed typical stramenopile flagella. The stramenopiles include the diatoms and the brown algae, which are photosynthetic, and the oomycetes, which are not. Other, smaller stramenopile groups include some that are nonphotosynthetic. Some botanists prefer to call the stramenopiles the "brown plant kingdom."

Diatoms are everywhere in the marine environment

Diatoms (Bacillar Eugknozoa

iophyta) are single-celled organisms, although some species associate in filaments. Many have sufficient carotenoids in their chloroplasts to give them a yellow or brownish color. All make chrysolaminarin (a carbohydrate) and oils as photosynthetic storage products. They lack flagella.

Architectural magnificence on a microscopic scale is the hallmark of the diatoms (Figure 27.18a). Many diatoms deposit silicon in their cell walls. The cell wall of some species

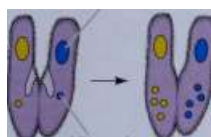
Alveola ta

Stramenopila

Rhodophyta

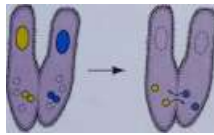
Chlorophyta

Macronucleus



Micronucleus

Q Two paramecia conjugate; all but one micronucleus in each cell disintegrate. The remaining micronucleus undergoes meiosis.

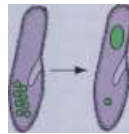


Q Three of the four haploid micronuclei disintegrate; the remaining micro-nucleus undergoes mitosis.

§ The paramecia donate micronuclei to each other. The macronuclei disintegrate.

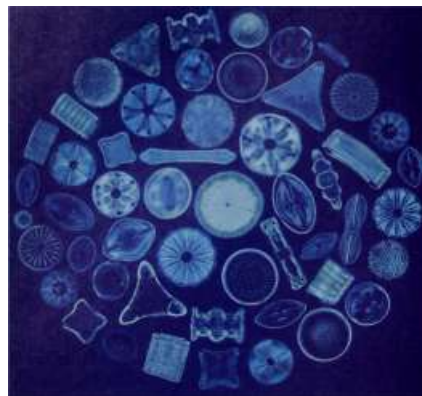


jj The micronuclei in each cell—each genetically different—fuse.



o The new diploid nuclei divide mitotically, eventually giving rise to a macronucleus and the appropriate number of micronuclei.

488 CHAPTER TWENTY-SEVEN



27.18 Diatom Diversity

(a) Diatoms exhibit a splendid variety of species-specific forms.

(b) This artificially colored scanning electron micrograph shows the intricate patterning of diatom cell walls.



7 urn

(a)

30 urn

is constructed in two pieces, with the wall of the top overlapping the wall of the bottom like the top and bottom of a petri plate. The silicon-impregnated walls have intricate, unique patterns (Figure 27.18b). Despite their remarkable morphological diversity, however, all diatoms are symmetrical—either bilaterally (with "right" and "left" halves) or radially (with the type of symmetry possessed by a circle).

Diatoms reproduce both sexually and asexually. Asexual reproduction is by cell division and is somewhat constrained by the stiff, silica-containing cell wall. Both the top and the bottom of the "petri plate" become tops of new "plates" without changing

appreciably in size; as a result, the new cells made from former bottoms are smaller than the parent cells (Figure 27.19). If this process continued indefinitely, one cell line would simply vanish, but sexual reproduction largely solves this potential problem. Gametes are formed, shed their cell walls, and fuse. The resulting zygote then increases substantially in size before a new cell wall is laid down.

Diatoms are everywhere in the marine environment and are frequently present in great numbers, making them major photosynthetic producers in coastal waters. Diatoms are also common in fresh water. Because the silicon-containing walls of dead diatom cells resist de-

composition, certain sedimentary rocks are composed al-

■t entirely of diatom skeletons that sank to the seafloor

over time. Diatomaceous earth, which is obtained from

Q Silicon-impregnated cell walls, shown edge-on, are two-part "Petri plates."

Growth of cell

such rocks, has many industrial uses, such as insulation, filtration, and metal polishing. It has also been used as an "Earth-friendly" insecticide that clogs the tracheae (breathing structures) of insects.

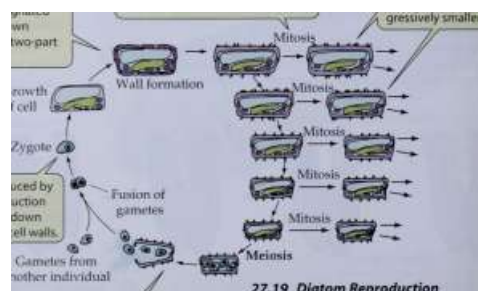
The brown algae include the largest protists

All the brown algae (Phaeophyta) are multicellular and composed either of branched filaments (Figure 27.20) or of leaflike growths called thalli (singular thallus) (Figure 27.21f). The brown algae obtain their namesake color from the carotenoid fucoxanthin, which is abundant in their chloro-

fj In asexual reproduction, the two parts of the cell wall separate, each becoming the top of a new "plate."

§JThe offspring cells from the bottom parts become progressively smaller.

Q Zygotes produced by sexual reproduction grow and lay down new full-size cell walls.



<3 Gametes from another individual

T

The "petri plate" splits and releases gametes.

27.19 Diatom Reproduction

Half of the cells created by asexual reproduction are smaller than the parent cells. Sexual reproduction creates new parent cells with full-sized cell walls.



(<j) Hormosira banksii

Ectocarpus sp.

27.20 Brown Algae

(a) A filamentous brown alga growing in Australia. This species is sometimes called "Neptune's necklace." (b) A filamentous brown alga seen through a light microscope.

plasts. The combination of this yellow-orange pigment with the green of chlorophylls a and c yields a brownish tinge.

The brown algae include the largest of the protists. Giant kelps, such as those of the genus *Macrocystis*, may be up to 60 meters long (see Figure 27.1c). The brown algae are almost exclusively marine. Some float in the open ocean; the most famous example is the genus *Sargassum*, which forms dense mats of vegetation in the Sargasso Sea in the mid-Atlantic. Most brown algae, however, are attached to rocks near the shore. A few thrive only where they are regularly exposed to heavy surf; a notable example is the sea palm *Postelsia palmaeformis* of the Pacific coast (Figure 27.21a). All

PROTISTS AND THE DAWN OF THE EUKARYA 489

of the attached forms develop a specialized structure, called a holdfast, that literally glues them to the rocks (Figure 27.21b).

Some brown algae differentiate extensively into stemlike stalks and leaflike blades, and some develop gas-filled cavities or bladders. For biochemical reasons that are only poorly understood, these gas cavities often contain as much as 5 percent carbon monoxide—a concentration high enough to kill a human. In addition to organ differentiation, the larger brown algae also exhibit considerable tissue differentiation. Most of the giant kelps have photosynthetic filaments only in the outermost regions of their stalks and blades. Within the photosynthetic region lie filaments of long cells that closely resemble the food-conducting tissue of plants. Called trumpet cells because they have flaring ends, these tubes rapidly conduct the products of photosynthesis through the body of the organism.

The cell walls of brown algae may contain as much as 25 percent alginic acid, a gummy polymer of sugar acids. Alginic acid cements cells and filaments together and provides good holdfast glue. It is used commercially as an emulsifier in ice cream, cosmetics, and other products.

Many protist and all plant life cycles feature alternation of generations

Brown algae, like many photosynthetic protists and all plants, exhibit a type of life cycle known as alternation of generations, in which a multicellular, diploid, spore-producing organism gives rise to a multicellular, haploid, gamete-producing organism. When two gametes fuse (a process called syngamy), a diploid organism is formed (Figure 27.22). The haploid organism, the diploid organism, or both may also reproduce asexually.

(a) *Postelsia palmaeformis*

The leaflike structures are the thalli of sea palm.

(b)



27.21 Brown Algae in a Turbulent Environment

Brown algae growing in the intertidal zone on an exposed rocky shore take a tremendous pounding by the surf, (a) Sea palm growing along the California coast. (b) The tough, branched holdfast that anchors the sea palm.

490 CHAPTER TWENTY-SEVEN

Q Spores germinate and divide by mitosis to make a multicellular haploid organism.



27.22 Alternation of Generations

In many multicellular photosynthetic protists and all plants, a diploid generation that produces spores alternates with a haploid generation that produces gametes.

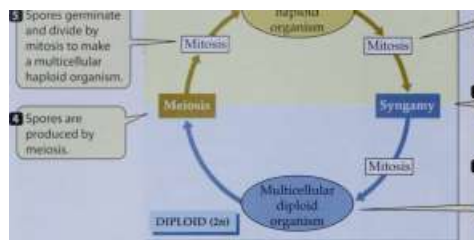
The two generations (spore-producing and gamete-producing) differ genetically (one has haploid cells and the other has diploid cells), but they may or may not differ morphologically. In heteromorphic alternation of generations, the two generations differ morphologically; in isomorphic alternation of generations they do not, despite their genetic difference. We will see examples of both heteromorphic and isomorphic alternation of generations in some representative brown and green algae. In discussing the life cycles of plants and multicellular photosynthetic protists, we will use the terms sporophyte ("spore plant") and gametophyte ("gamete plant") to refer to the multicellular diploid and haploid generations, respectively.

Gametes are not produced by meiosis because the gamete-producing generation is already haploid. Instead, specialized cells of the diploid sporophyte, called sporocytes, divide meiotically to produce four haploid spores. The spores may eventually germinate and divide mitotically to produce multicellular haploid gametophytes, which produce gametes by mitosis and cytokinesis.

Gametes, unlike spores, can produce new organisms only by fusing with other gametes. The fusion of two gametes produces a diploid zygote, which then undergoes mitotic divisions to produce a diploid organism: the sporophyte generation. The sporocytes of the sporophyte generation then undergo meiosis and produce haploid spores, starting the cycle anew.

The brown algae exemplify the extraordinary diversity found among the photosynthetic protists. One genus of simple brown algae is *Ectocarpus* (see Figure 27.20b). Its branched filaments, a few centimeters long, commonly grow on shells and stones. The gametophyte and spore-generations of *Ectocarpus* can be distinguished only by chromosome number or reproductive products (spores or gametes). Thus the generations are isomorphic.

U MAI



Q Gametes (n) are T produced by ^^^ mitosis of — * :::: ^ "1 haploid cells.

Gametes fuse to form a zygote.

The zygote develops into a multicellular i diploid organism.

By contrast, some kelps of the genus *Laminaria* and some other brown algae show a more complex heteromorphic alternation of generations. The larger and more obvious generation of these species is the sporophyte. Meiosis in special fertile regions of the leaflike fronds produces haploid zoospores—motile spores that are propelled by flagella. These germinate to form a tiny, filamentous gametophyte that produces either eggs or sperm. The eggs and sperm of brown algae typically have flagella.

The oomycetes include water molds and their relatives

A nonphotosynthetic stramenopile group called oomycetes consists in large part of the water molds and their terrestrial relatives, such as the downy mildews. Water molds are filamentous and stationary, and they feed by absorption. If you have seen a whitish, cottony mold growing on dead fish or dead insects in water, it was probably a water mold of the common genus *Saprolegnia* (Figure 27.23).

The oomycetes are coenocytes: They have many nuclei enclosed in a single plasma membrane. Their filaments have no cross-walls to separate the many nuclei into discrete cells. Their cytoplasm is continuous throughout the body of the mold, and there is no single structural unit with a single nucleus, except in certain reproductive stages. A distinguishing feature of the oomycetes is their flagellated reproductive cells. Oomycetes are diploid throughout most of their life cycle and have cellulose in their cell walls.

The water molds, such as *Saprolegnia*, are all aquatic and saprobic (they feed on dead organic matter). Some other



Saprolegnia sp.

27.23 An Oomycete

The filaments of a water mold radiate from the carcass of an insect.

oomycetes are terrestrial. Although most terrestrial oomycetes are harmless or helpful decomposers of dead matter, a few are serious plant parasites that attack crops such as avocados, grapes, and potatoes. The mold *Plasmopara infestans*, for example, is the causal agent of late blight of potatoes, which brought about the great Irish potato famine of 1845-1847. *P. infestans* destroyed the entire Irish potato crop in a matter of days in 1846. Among the consequences of the famine were a million deaths from starvation and the emigration of about 2 million people, mostly to the United States.

Rhodophyta

Almost all red algae (Rhodophyta) are multicellular (Figure 27.24). Some botanists now refer to the red algae as the "red plant kingdom." Their characteristic color is a result of the photosynthetic pigment phycoerythrin, which is found in relatively large amounts in the chloroplasts of many species. In addition to phycoerythrin, red algae contain phycocyanin, carotenoids, and chlorophyll.

The red algae include species that grow in the shallowest tide pools, as well as the algae found deepest in the ocean (as deep as 260 meters if nutrient conditions are right and the water is clear enough to permit the penetration of light). Very few red algae inhabit fresh water. Most grow attached to a substrate by a holdfast.

In a sense the red algae, like several other groups of algae, are misnamed. They have the capacity to change the relative amounts of their various photosynthetic pigments depending on the light conditions where they are growing. Thus the leaflike *Chondrus crispus*, a common North Atlantic red alga, may appear bright green when it is growing at or near the surface of the water and deep red when growing at greater depths. The ratio of pigments present

Euglenozoa

Alveolata



Chlorophyta



depends to a remarkable degree on the intensity of the light that reaches the alga. In deep water, where the light is dimmest, the alga accumulates large amounts of phycoerythrin, an accessory photosynthetic pigment (see Figure 8.7). Algae in deep water have as much chlorophyll as the green ones near the surface, but the accumulated phycoerythrin makes them look red.

In addition to being the only photosynthetic protists with phycoerythrin and phycocyanin among their pigments, the red algae have two other unique characteristics: They store the products of photosynthesis in the form of floridean starch, which is composed of very small, branched chains of approximately 15 glucose units. And they produce no motile, flagellated cells at any stage in their life cycle. The male gametes lack cell walls and are slightly amoeboid; the female gametes are completely immobile.

Some red algal species enhance the formation of coral reefs. Like the coral animals, they possess the biochemical machinery for depositing calcium carbonate both in and around their cell walls. After the death of the corals and algae, the calcium carbonate persists, sometimes forming substantial rocky masses.

Some red algae produce large amounts of mucilaginous polysaccharide substances, which contain the sugar galactose with a sulfate group attached. This material readily forms solid gels and is the source of agar, a substance widely used in the

laboratory for making a solid aqueous medium on which tissue cultures and many microorganisms can be grown.

Certain red algae became endosymbionts, long ago, within the cells of other, nonphotosynthetic protists, eventually giving rise to chloroplasts. This was the evolutionary origin of the distinctive chloroplasts of the photosynthetic stramenopiles (the brown algae and the diatoms).



(a) *Palmaria palmata*

(b) *Poh/siphonia* sp.

27.24 Red Algae

(a) Dulse, a large, edible red alga, is growing here on rocks in New Brunswick, Canada, (b) Both vegetative and reproductive structures of this alga can be seen under the light microscope.

492 CHAPTER TWENTY-SEVEN

Chlorophyta

The "green algae" do not form a monophyletic group, but include at least two lineages. One major lineage constitutes the Chlorophyta, a monophyletic group. A sister lineage to the Chlorophyta consists of other green algal lineages and the plant kingdom. The green algal lineages share characters that distinguish them from other protists: Like the plants, they contain chlorophylls a and b, and their reserve of photosynthetic products is stored as starch in plastids. There are more than 17,000 species of chlorophytes. Most chlorophytes are aquatic—some are marine, but more are freshwater forms—but others are terrestrial, living in moist environments. The chlorophytes range in size from microscopic unicellular forms to multicellular forms many centimeters in length.

Chlorophytes vary in shape and cellular organization

We find in the Chlorophyta an incredible variety in shape and construction of the algal body. *Chlorella* is an example of the simplest type: unicellular and flagellated.

Surprisingly large and well-formed colonies of cells are found in such freshwater groups as the genus *Volvox*. These cells are not differentiated into tissues and organs, as in

Parent Somatic (a) *Volvox* sp. colony cells

Alveolata

Stramenopila

Rhodophyta



Specialized reproductive cells produce and release daughter colonies.

(c) *Micrasterias* sp.

wm



plants and animals, Euglenozoa

but the colonies show vividly how the preliminary step of this great evolutionary development might have been taken. In *Volvox*, the origins of cell specialization can be seen as certain cells within the colony (Figure 27.25a) are specialized for reproduction.

While *Volvox* is colonial and spherical, *Oedogonium* is multicellular and filamentous, and each of its cells has only one nucleus. *Cladophora* is multicellular, but each cell is multinucleate. *Bryopsis* is tubular and coenocytic, forming cross-walls only when reproductive structures form. *Acetabularia* is a single, giant uninucleate cell a few centimeters long that becomes multinucleate only at the end of the reproductive stage. *Ulva lactuca* is a membranous sheet two cells thick; its unusual appearance justifies its common name: sea lettuce (Figure 27.25b).

Chlorophyte life cycles are diverse

The life cycles of chlorophytes show great diversity. Let's examine two chlorophyte life cycles in detail, beginning with that of the sea lettuce *Ulva lactuca* (Figure 27.26). The diploid sporophyte of this common seashore organism is a thin cellular sheet a few centimeters in diameter. Some of its cells (sporocytes) differentiate and undergo meiosis and cytokinesis, producing motile haploid spores (zoospores). These swim away, each propelled by four flagella, and some eventually find a suitable place to settle. The spores then lose their fla-

Semicells

Isthmus



4 (im

27.25 Chlorophytes

(a) *Volvox* colonies are precisely spaced arrangements of cells. Several daughter colonies can be seen within the parent colonies. (b) Submerged in a tidal pool, this large sea lettuce appears "leafy" but is actually made up of membranous sheets of cells. (c) Each of these "constrict-ed" desmids is a microscopic, unicellular alga comprising two semicells. The central isthmus contains the cell's nucleus. A single large, ornate chloroplast fills much of the volume of each semicell.

, Haploid

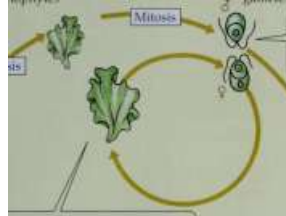
HAPLOID (n) | gametocytes

Haploid (n) gametes

Haploid spores

Mitosis

•r



Male and female gametes look the same—*Ulva* is isogamous.



Fusing gametes

The diploid sporophytes and haploid gametophytes look alike—the life cycle is isomorphic.

Syngamy

7

/

Mitosis

Diploid (2n) zygote

flagella, undergo mitosis, and produce a new gametophyte directly; in other words, the gametes can also function as zoospores. Few chlorophytes other than *Ulva* have motile gametes that can also function as zoospores.

In contrast to the isomorphic life cycle of *Ulva*, many other chlorophytes have a heteromorphic life cycle: Sporophyte and gametophyte generations differ in structure. In one variation of the heteromorphic life cycle—the haplontic life cycle (Figure 27.27)—a multicellular haploid individual produces gametes that fuse to form a zygote. The zygote functions directly as a sporophyte, undergoing meiosis to produce spores, which in turn produce a new haploid individual. In the entire haplontic life cycle, only one cell—the zygote—is diploid. The filamentous organisms of the genus *Ulothrix* are examples of haplontic chlorophytes.

Other chlorophytes have a diplontic life cycle like that of many animals. In a diplontic life cycle, meiosis of sporocytes

rn



27.26 An Isomorphic Life Cycle

The life cycle of *Ulva lactuca* is an example of isomorphic alternation of generations.

gella and begin to divide mitotically, producing a thin filament that develops into a broad sheet only two cells thick. The gametophyte thus produced looks just like the sporophyte—in other words, *Ulva* has an isomorphic life cycle.

An individual gametophyte can produce only male or female gametes—never both. The gametes arise mitotically within single cells (called gametangia), rather than within a specialized multicellular structure, as in plants. Both types of gametes bear two flagella (in contrast to the four flagella of a haploid spore) and hence are motile.

In most species of *Ulva* the female and male gametes are indistinguishable structurally, making those species isogamous — having gametes of identical appearance. Other chlorophytes, including some other species of *Ulva*, are anisogamous — having female gametes that are distinctly larger than the male gametes.

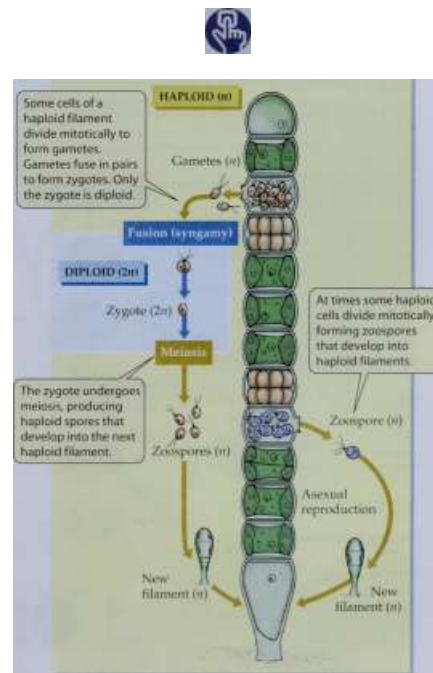
Female and male gametes come together and unite, losing their flagella as the zygote forms and settles. After resting briefly, the zygote begins mitotic division, producing a multicellular sporophyte. Any gametes that fail to find partners can settle

down on a favorable substrate, lose their

27.27 A Haplontic Life Cycle

In the life cycle of *Ulothrix*, a filamentous, multicellular gametophyte generation alternates with a sporophyte generation consisting of a single cell.

Some cells of a haploid filament divide mitotically to form gametes. Gametes fuse in pairs to form zygotes. Only the zygote is diploid.



494 CHAPTER TWENTY-SEVEN

produces gametes directly; the gametes fuse, and the resulting zygote divides mitotically to form a new multicellular sporophyte. In such organisms, every cell except the gametes is diploid. Between these two extremes are chloro-phytes whose gametophyte and sporophyte generations are both multicellular, but that have one phase (usually the sporophyte) that is much larger and more prominent than the other.

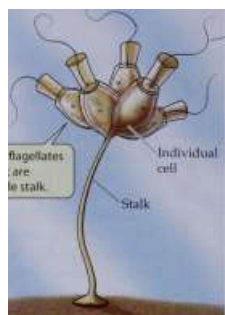
There are green algae other than chlorophytes

As we mentioned above, the Chlorophyta are the largest lineage of green algae, but there are other green algal lineages as well. Those lineages are branches of a lineage that also includes the plant kingdom. The green algal lineage that is sister to the plant kingdom, a group of organisms called charophytes, will be described in the next chapter. But now let's consider a close protist relative of the animals.

Choanoflagellida

One group of protists with flagella, the Choanoflagellida, is thought to comprise the closest relatives of the animals. Members of this group are colonial (Figure 27.28) and are thought to be closely related to the sponges, the most ancient of the surviving phyla of animals. Sponges are also colonial rather than truly multicellular, in that they lack organized tissues and their cells can be separated and recom-bined. Choanoflagellates bear a striking resemblance to the most characteristic type of cell found in the sponges (compare Figures 27.28 and 31.4).

Individual choanoflagellates form colonies that are attached to a single stalk.



Flagellum

27.28 A Link to the Animal Kingdom

This colonial choanoflagellate may be a close relative of the sponges and thus a link between protists and the kingdom Animalia. The connection of unicellular organisms into colonies often leads to the evolution of specialized cells and true multicellularity; another example of a colonial protist was seen earlier in Volvox (Figure 27.25a).

A History of Endosymbiosis

As we have already seen, many protists possess chloroplasts. Groups with chloroplasts appear in several distantly related protist lineages. Some of these groups differ from others in the photosynthetic pigments their chloroplasts contain. And we've seen that not all chloroplasts have a pair of surrounding membranes—in some protists, they

PRIMARY ENDOSYMBIOSIS

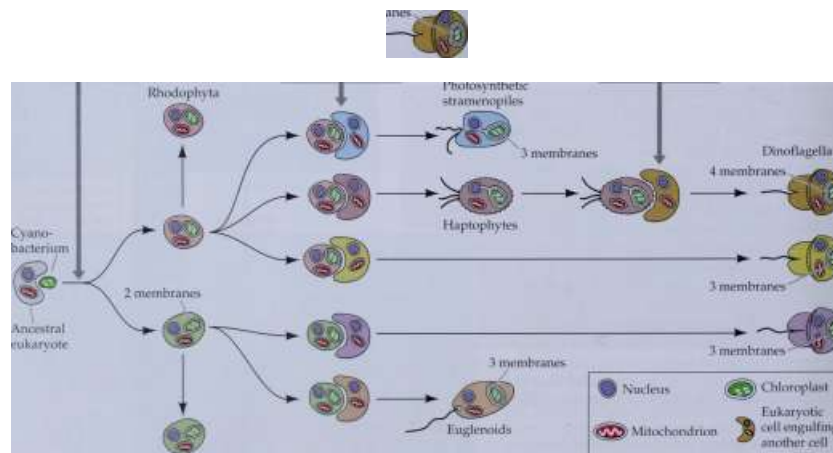
SECONDARY ENDOSYMBIOSIS

Rhodophyta

Photosynthetic stramenopiles

TERTIARY ENDOSYMBIOSIS

Dinoflagellates 4 membranes.



Ancestral eukaryote

3 membranes



Euglenoids

^) Nucleus (f2K) Mitochondrion

Chloroplast

Eukaryotic cell engulfing another cell

Chlorophyta, other green algae, and plants

rn ^ 27.29 A Chloroplast Family Tree

One or two primary endosymbioses followed by secondary and tertiary endosymbioses gave rise to all of today's chloroplasts.



have three membranes. We now understand these observations in terms of a remarkable series of endosymbioses.

All chloroplasts trace back to the engulfment of an ancestral cyanobacterium by a larger eukaryotic cell (Figure 27.29). This event constituted primary endosymbiosis. The cyanobacterium had a single membrane—its plasma membrane—and that membrane was surrounded by part of the eukaryote's plasma membrane that wrapped around the bacterium as it was taken up. Thus, the original chloroplasts had two surrounding membranes.

Primary endosymbiosis gave rise to the chloroplasts of the green algae and the red algae. We do not yet know whether both trace back to a single primary endosymbiosis, with later divergence, or whether they resulted from independent occurrences of primary endosymbiosis. In either case, each line participated in further endosymbioses.

The photosynthetic euglenoids derived their chloroplasts from secondary endosymbiosis. Their ancestor took up a unicellular chlorophyte, retaining the endosymbiont's chloroplast and eventually losing the rest of its constituents. This history

accounts for the fact that the photosynthetic euglenoids have the same photosynthetic pigments as the chloro-phytes and plants. It also accounts for the third membrane of the euglenoid chloroplast, which is derived from the eu-glenoid's plasma membrane.

Other photosynthetic protist groups Common derived their chloroplasts by endosym- ancestor biosis with unicellular red algae. Both the green lineage and the red lineage of chloroplasts appear to have given rise to more than one secondary endosymbiosis. At least one secondary endosymbiosis produced a unicellular protist that became, itself, a partner in a tertiary endosymbiosis!

Although euglenoid chloroplasts are descendants of a chlorophyte, and stramenopile chloroplasts are descendants of a red alga, this does not mean that euglenoids themselves are descendants of a chlorophyte, nor are stramenopiles themselves descendants of a red alga. The ancestors that took up green or red algae in secondary endosymbiosis had their own evolutionary histories. It has taken much research to piece together the lineages as we now understand them.

The monophyletic groups of protists that we have discussed are summarized in Table 27.1 and Figure 27.9. Now let's consider two major types of protist body forms—amoebas and other organisms with pseudopods, and slime molds—that are not monophyletic. Rather, they have reappeared in various branches of the eukaryote family tree.

Some Recurrent Body Forms

Amoebas used to be classified together in a single protist group. However, the amoeba body plan has popped up again and again in the course of the evolution of the Eukarya (Figure 27.30). Similarly, three kinds of organisms called slime molds, once classified together, may be quite different phylogenetically

Amoebas form pseudopods

The pseudopods used by amoebas for locomotion are a hallmark of these protists. This body plan has appeared by convergent evolution in various protist groups. The mechanism of amoeboid motion will be discussed in Chapter 47.

Amoebas have often been portrayed in popular writing as blobs—the simplest form of "animal" life imaginable. Superficial examination of a typical amoeba shows how such an impression might have been obtained. An amoeba consists of a single cell. It feeds on small organisms and particles of organic matter by phagocytosis, engulfing them with its pseudopods.

But amoebas are specialized protists. Many are adapted for life on the bottoms of lakes, ponds, and other bodies of water. Their creeping locomotion and their manner of engulfing food particles fit them for life close to a relatively rich supply of sedentary organisms or organic particles. Most amoebas exist as predators, parasites, or scavengers. A few are photosynthetic.

Amoebas of the free-living genus *Naegleria*, some of which can enter humans and cause a fatal disease of the nervous system, have a two-stage life cycle, one stage having amoeboid cells and the other flagellated cells. Some

BACTERIA

ARCHAEA

Amoebas

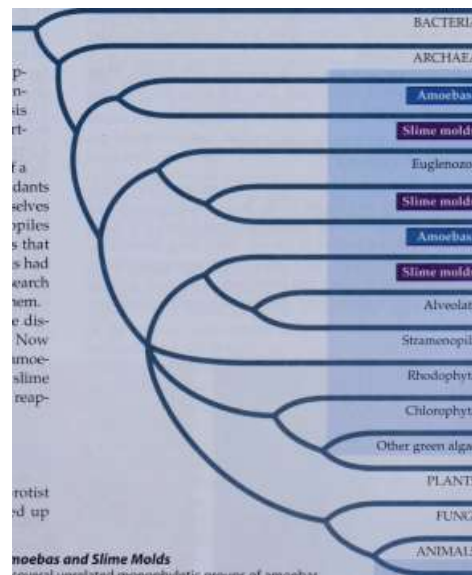
Slime molds

Euklenozoa

Slime molds

Amoebas

Slime molds



Alveolata

Stramenopila

Rhodophyta

Chlorophyta

Other green algae

PLANTS

27.30 Amoebias and Slime Molds

There are several unrelated monophyletic groups of amoebas and slime molds. Body forms featuring amoeboid pseudopods have evolved repeatedly, as have slime molds.

Choano-flagellida

496 CHAPTER TWENTY-SEVEN

amoebas are shelled, living in casings of sand grains glued together (see Figure 27.7b) or in shells secreted by the organism itself.

Actinopods have thin, stiff pseudopods

The actinopods are recognizable by their thin, stiff pseudo-pods, which are reinforced by microtubules. The pseudopods play at least four roles:

- ▶ They greatly increase the surface area of the cell for exchange of materials with the environment.
- ▶ They help the cell float in its marine or freshwater environment.
- ▶ They provide locomotion in some species.
- ▶ They are the cell's feeding organs, trapping smaller organisms and often taking them up by endocytosis.

Radiolarians, actinopods that are exclusively marine, are perhaps the most beautiful of all microorganisms (Figure 27.31a). Almost all radiolarian species secrete glassy endoskeletons (internal skeletons) from which needlelike pseudopods project. Part of the skeleton is a central capsule within the cytoplasm. The skeletons of the different species are as varied as snowflakes, and many have elaborate geometric designs. A few radiolarians are among the largest of the unicellular protists, with skeletons measuring several millimeters across. Innumerable radiolarian skeletons, some as old as 700 million years, form the sediments under some tropical seas.

Heliozoans lack an endoskeleton (Figure 27.31b). Most heliozoans live in fresh water. They roll along the substrate by shortening and elongating their pseudopods.

Foraminiferans have created vast limestone deposits

Foraminiferans are marine protists that secrete shells of calcium carbonate (see Figure 27.7a). Some foraminiferans live as plankton (free-floating microscopic organisms), and many others live at the bottom of the sea. Their long, threadlike, branched pseudopods reach out through numerous microscopic pores in the shell and interconnect to create a sticky net, which the foraminiferan uses to catch smaller plankton.

After foraminiferans reproduce (by mitosis and cytokinesis), the daughter cells abandon the parent shell and make new shells of their own. The discarded skeletons of ancient foraminiferans make up extensive limestone deposits in various parts of the world, forming a layer hundreds to thousands of meters deep over millions of square kilometers of ocean bottom. Foraminiferan skeletons also make up the sand of some beaches. A single gram of such sand may contain as many as 50,000 foraminiferan shells.

The shells of individual foraminiferan species have distinctive shapes and are easily preserved as fossils in marine sediments. Each geological period has distinctive foraminiferan species. For this reason, and because they are so abundant, the remains of foraminiferans are especially valuable as indicators in the classification and dating of sedimentary rocks, as well as in oil prospecting.

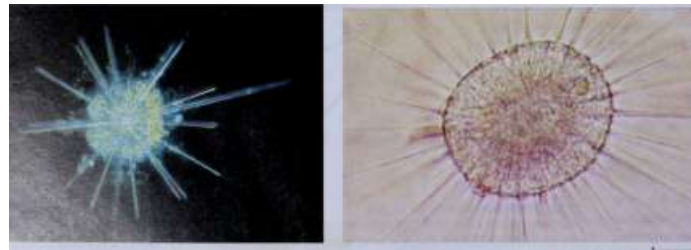
Slime molds release spores from erect fruiting bodies

The three groups of slime molds seem so similar at first glance that they were once grouped in a single phylum. However, the slime molds are actually so different that some biologists now classify them in different kingdoms. We will consider two of these groups, called acellular slime molds and cellular slime molds.

The slime molds share only general characteristics. All are motile, all ingest particulate food by endocytosis, and all form spores on erect fruiting bodies. They undergo striking changes in organization during their life cycles, and one stage consists of isolated cells that engage in absorptive nutrition. Some slime molds may cover areas of 1 meter or more in diameter while in their less aggregated stage. Such a large slime mold may weigh more than 50 grams. Slime molds of both types favor cool, moist habitats, primarily in forests. They range from colorless to brilliantly yellow and orange.

ACELLULAR SLIME MOLDS FORM MULTINUCLEATE MASSES. If the

nucleus of an amoeba began rapid mitotic division, accompanied by a tremendous increase in cytoplasm and organelles, the resulting organism might resemble the acel-



Radiolaridn (species not identified)

(b) Actinosphaerium eichorni

1 50 |im

27.37 Actinopods

(a) A radiolarian displays its intricate glassy skeleton, (b) A heliozoan with long pseudopods.



(a) Physarum poly,

27.32 Acellular Slime Molds

(a) Plasmodia of yellow slime mold cover a rock in Nova Scotia

(b) The fruiting structures—sporangiophores (yellow) and sporangia (black)—of Physarum.

(b) Physarum sp.

1 mm

ular slime molds (Myxomycota). During its vegetative (feeding) phase, an acellular slime mold is a wall-less mass of cytoplasm with numerous diploid nuclei. This mass streams very slowly over its substrate in a remarkable network of

strands called a plasmodium* (Figure 27.32a). The Plasmodium of an acellular slime mold is an example of a coenocyte, a body in which many nuclei are enclosed in a single plasma membrane. The outer cytoplasm of the plasmodium (closest to the environment) is normally less fluid than the interior cytoplasm and thus provides some structural rigidity.

Acellular slime molds such as *Physarum* (a popular research subject) provide a dramatic example of movement by cytoplasmic streaming. The outer cytoplasmic region becomes more fluid in places, and cytoplasm rushes into those areas, stretching the plasmodium. This streaming somehow reverses its direction every few minutes as cytoplasm rushes into a new area and drains away from an older one, moving the plasmodium over its substrate in search of food. Sometimes an entire wave of plasmodium moves across the substrate, leaving strands behind. Actin filaments and a contractile protein called myxomyosin interact to produce the streaming movement. As it moves, the plasmodium engulfs food particles—predominantly bacteria, yeasts, spores of fungi, and other small organisms, as well as decaying animal and plant remains.

An acellular slime mold can grow almost indefinitely in its plasmodial stage, as long as the food supply is adequate and other conditions, such as moisture and pH, are favorable. However, one of two things can happen if conditions become unfavorable. First, the plasmodium can form an irregular mass of hardened cell-like components called a sclerotium. This resting structure rapidly becomes a plasmodium again when favorable conditions are restored.

Alternatively, the plasmodium can transform itself into

*Do not confuse the plasmodium of an acellular slime mold with the genus *Plasmodium*, the apicomplexan that is the cause of malaria.

spore-bearing fruiting structures (Figure 27.32b). These stalked or branched structures, called sporangiophores, rise from heaped masses of plasmodium. They derive their rigidity from walls that form and thicken between their nuclei. The nuclei of the plasmodium are diploid, and they divide by meiosis as the sporangiophore develops. One or more knobs, called sporangia, develop on the end of the stalk. Within a sporangium, haploid nuclei become surrounded by walls and form spores. Eventually, as the sporangiophore dries, it sheds its spores.

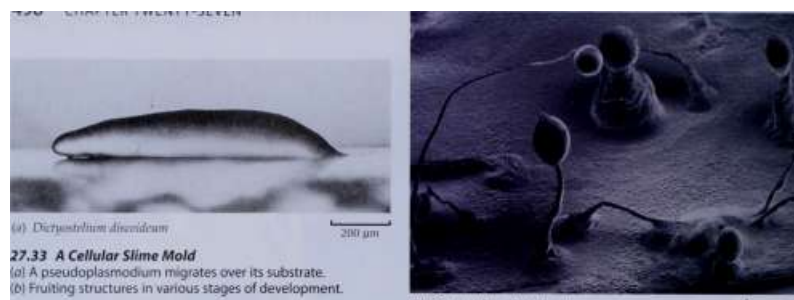
The spores germinate into wall-less, flagellated, haploid cells called swarm cells, which can either divide mitotically to produce more haploid swarm cells or function as gametes. Swarm cells can live as separate individual cells, and can become walled and resistant resting cysts when conditions are unfavorable. When conditions improve again, the cysts release flagellated swarm cells. Two swarm cells can also fuse to form a diploid zygote, which divides by mitosis (but without a wall forming between the nuclei) and thus forms a new, coenocytic plasmodium.

CELLS RETAIN THEIR IDENTITY IN THE CELLULAR SLIME MOLDS.

Whereas the plasmodium is the basic vegetative unit of the acellular slime molds, an amoeboid cell is the vegetative unit of the cellular slime molds. Large numbers of cells called myxamoebas, which have single haploid nuclei, engulf bacteria and other food particles by endocytosis and reproduce by mitosis and fission. This simple life cycle stage, consisting of swarms of independent, isolated cells, can persist indefinitely as long as food and moisture are available.

When conditions become unfavorable, however, the cellular slime molds aggregate and form fruiting structures, as do their acellular counterparts. The apparently independent myxamoebas aggregate into a mass called a slug or pseudoplasmodium (Figure 27.33a). Unlike the true plasmodium of the acellular slime molds (see Figure 27.32rt), this structure is not simply a giant sheet of cytoplasm with many nuclei; the individual myxamoebas retain their plasma membranes and, therefore, their identity.

498 CHAPTER TWENTY-SEVEN



(<?) Dictyostelium discoideum

27.33 A Cellular Slime Mold

(a) A pseudoplasmodium migrates over its substrate. (fc>) Fruiting structures in various stages of development

(b) Dictyostelium discoideum

500 urn

The chemical signal that causes the myxamoebas of cellular slime molds to aggregate into a slug is 3',5'-cyclic adenosine monophosphate (cAMP), a compound that plays many important roles in chemical signaling in animals (see Chapter 15). A slug may migrate over its substrate for several hours before becoming motionless and reorganizing to construct a delicate, stalked fruiting structure (Figure 27.33b). Cells at the top of the fruiting structure develop into thick-walled spores, which

are eventually released. Later, under favorable conditions, the spores germinate, releasing myxamoebas.

The cycle from myxamoebas through slug and spores to new myxamoebas is asexual. Cellular slime molds also have a sexual cycle, in which two myxamoebas fuse. The product of this fusion develops into a spherical structure that ultimately germinates, releasing new haploid myxamoebas.

In the remaining chapters of Part Four, we will explore the three classic kingdoms of multicellular eukaryotes. Chapters 28 and 29 deal with the kingdom Plantae (which, combined with the Chlorophyta and other green algae, is called by some botanists the "green plant kingdom"). Chapter 30 presents the kingdom Fungi, and Chapters 31-33 describe the kingdom Animalia. These kingdoms all arose from protist ancestors.

Chapter Summary

Protists Defined

► In this book we define the protists simply as all eukaryotes that are not plants, fungi, or animals. The protists are not a monophyletic group.

The Origin of the Eukaryotic Cell

► The modern eukaryotic cell arose from an ancestral prokaryote in several steps. Probable steps included loss of the cell wall and inward folding of the plasma membrane. Review Figure 27.2

► In subsequent steps, an infolded plasma membrane attached to the chromosome may have led to the formation of a nuclear envelope. A primitive cytoskeleton evolved. Review Figure 27.3

► The first truly eukaryotic cell was larger than its prokaryote ancestor, and it may have possessed one or more flagella of the eukaryotic type.

► The incorporation of prokaryotic cells as endosymbionts gave rise to eukaryotic organelles. Peroxisomes, which protected the host cell from an oxygen-rich atmosphere, may have been the first organelles of endosymbiotic origin. Mitochondria evolved from once free-living proteobacteria, and chloroplasts evolved from once free-living cyanobacteria. Cells with nuclei probably appeared before the first cells with mitochondria. Review Figure 27.3

General Biology of the Protists

► Most protists are aquatic; some live within other organisms. The great majority are unicellular and microscopic, but many are multicellular and a few are enormous.

► "Protozoan" is an outdated term sometimes applied to protists, mostly ingestive heterotrophs, that were once classified as animals. "Alga" is an outdated term sometimes applied to photosynthetic protists.

► Protists vary widely in their modes of nutrition, metabolism, and locomotion. Some protist cells contain contractile vacuoles, and some digest their food in food vacuoles. Review Figures 27.5, 27.6

► Protists have a variety of cell surfaces, some of them protective. Review Figure 27.7

► Many protists contain endosymbiotic prokaryotes. Some protists are endosymbionts in other cells, including other protists. Some endosymbiotic protists perform photosynthesis, to the advantage of their hosts.

► Most protists reproduce both asexually and sexually.

Protist Diversity

► Molecular and other techniques are enabling biologists to identify many monophyletic groups of protists. Review Figure 27.9 and Table 27.1

Euglenozoa

► The Euglenozoa are a monophyletic group of unicellular protists with flagella.

► Euglenoids are Euglenozoa, such as *Euglena*, that are often photosynthetic and have anterior flagella.

► Kinetoplastids are Euglenozoa, such as *Trypanosoma*, that have a single, large mitochondrion, in which RNA is edited.

Alveolata

► The Alveolata are a monophyletic group of unicellular organisms with alveoli (cavities) beneath their plasma membranes.

► Dinoflagellates are marine alveolates with a golden-brown color that results from their photosynthetic and accessory

PROTISTS AND THE DAWN OF THE EUKARYA 499

pigments. They are major contributors to world photosynthesis. Many are endosymbionts; in that role they are important contributors to coral reef growth. Dinoflagellates are responsible for toxic "red tides."

► Apicomplexans are parasitic alveolates with an amoebalike body form. Their spores, containing a mass of organelles at the apical end, are adapted to the invasion of host tissue. The apicomplexan *Plasmodium*, which causes malaria, uses two alternate hosts (humans and *Anopheles* mosquitoes). Review Figure 27.13

► Ciliates are alveolates such as *Paramecium* that move rapidly by means of cilia and have two kinds of nuclei. The macronuclei control the cell by means of transcription and translation. The micronuclei are responsible for genetic recombination, accomplished by conjugation, which is sexual but not reproductive. Some ciliates have a remarkably complex internal structure. Review Figures 27.15, 27.16, 27.17

Stramenopila

► Stramenopiles typically have two flagella of unequal length, the longer bearing rows of tubular hairs. Some stramenopile groups are photosynthetic.

► Diatoms are unicellular stramenopiles, many of which have complex, two-part, glassy cell walls. They contribute extensively to world photosynthesis. Review Figure 27.19

► The brown algae are predominantly multicellular, photo-synthetic stramenopiles. They include the largest of all protists, and some show considerable tissue differentiation.

► In many multicellular photosynthetic protists and in all plants, both haploid and diploid cells undergo mitosis, leading to an alternation of generations. The diploid sporophyte generation forms spores by meiosis, and the spores develop into haploid organisms. This haploid gametophyte generation forms gametes by mitosis, and their fusion yields zygotes that develop into the next generation of sporophytes. Review Figure 27.22

► Oomycetes are a group of nonphotosynthetic stramenopiles including water molds and downy mildews. The oomycetes are coenocytic. They are diploid for most of their life cycle.

Rhodophyta

► Red algae (Rhodophyta) are multicellular, photosynthetic protists. They differ from the other photosynthetic protist groups in having a characteristic storage product (floridean starch) and lacking flagellated reproductive cells.

Chlorophyta

► The Chlorophyta, a monophyletic group of green algae, are often multicellular. Like plants, they contain chlorophylls a and b and use starch as a storage product. The chlorophytes have diverse life cycles; among these are the isomorphic alternation of generations of *Ulva* and the haplontic life cycle of *Ulothrix*. Review Figures 27.26, 27.27

► The chlorophytes are sister to a lineage that includes other green algae and the plant kingdom.

Choanoflagellida

► The Choanoflagellida are protists with flagella and a body type similar to the most characteristic type of cell found in sponges. The Choanoflagellida are sister to the animal kingdom.

A History of Endosymbiosis

* ■ Primary endosymbiosis of a cyanobacterium and a eukaryote gave rise to the chloroplasts of green algae, plants, and red algae. Review Figure 27.29

► Secondary endosymbiosis of eukaryotes with unicellular green or red algae gave rise to the chloroplasts of euglenoids, stramenopiles, and other groups. A cell of one of those groups, in tertiary endosymbiosis, has given rise to another type of chloroplast.

Some Recurrent Body Forms

► Some similar body forms are found in several different, unrelated protist groups. Review Figure 27.30

► Amoebas, which appear in many protist groups, move by means of pseudopods.

► Actinopods have thin, stiff pseudopods that serve various functions, including food capture.

► Foraminiferans also use pseudopods for feeding, and secrete shells of calcium carbonate.

► Acellular slime molds and cellular slime molds are superficially very similar, moving as slimy masses and producing stalked fruiting structures. However, they differ at the cellular level. Acellular slime molds are coenocytes with diploid nuclei. Cellular slime molds consist of individual haploid cells that aggregate into masses consisting of distinct cells.

For Discussion

1. For each type of organism below, give a single characteristic that may be used to differentiate it from the other, related organism(s) in parentheses.

a. foraminiferans (radiolarians)

- b. Euglena (Volvox)
 - c. Trypanosoma (Giardia)
 - d. Amoeba (flagellate)
 - e. Physarum (Dictyostelium)
2. For each of the following groups, give at least two characteristics used to distinguish the group from other groups.
 - a. Ciliophora
 - b. Apicomplexa
 - c. Phaeophyta
 - d. Rhodophyta
 3. In what sense are sex and reproduction independent of each other in the ciliates? What does that suggest as to the most important role of sex in biology?
 4. Why are dinoflagellates and apicomplexans placed in one group of protists and brown algae and oomycetes in another?
 5. Giant seaweed (mostly brown algae) have "floats" that aid in keeping their fronds suspended at or near the surface of the water. Why is it important that the fronds be suspended?
 6. Why are algal pigments so much more diverse than those of plants?
 7. For each of the groups Chlorophyta, Euglenozoa, and Rhodophyta, indicate how many membranes surround their chloroplasts, and offer a reasonable explanation in each case. Why do some dinoflagellates have more membranes around their chloroplasts than other dinoflagellates?



Plants without Seeds: From Sea to Land



HOW DO WE RUN OUR ENGINES, HEAT OUR homes, smelt our metals, and generate much of our electricity? We do these things by burning plant-based fuels. The great majority of such fuels—petroleum and natural gas, the so-called fossil fuels—comes from the remains of plants without seeds that grew in great forests hundreds of millions of years ago.

In some parts of the world, people derive the majority of their fuel from peat bogs. They harvest and burn peat, another substance produced largely by a nonseed plant.

Peat consists of partially decomposed plant material. It forms as rapidly growing upper layers of moss, primarily the genus *Sphagnum*, along with some other plants, compress the deeper-lying layers. Peatlands cover an area approximately half as large as the United States—more than 1 percent of Earth's total surface.

Sphagnum is one of the most abundant plants on Earth, yet it and its mossy neighbors at first glance seem to lack adaptations to life on land. Mosses have no internal "plumbing system" to move water and nutrients within their bodies, and their leafy photosynthetic organs are only one cell thick. They require liquid water in order to reproduce, and indeed seem at first glance to be highly dependent on external moisture. They can dry out to the point of becoming brittle, yet they snap back as soon as a bit of water is available. How do they manage to survive on land?

That mosses and their relatives do have effective adaptations for life in terrestrial environments is obvious from their wide distribution. Most live in moist habitats, but a few mosses even live in deserts.

Earth did not take on a green tint until about half a billion years ago, long after the ancestors of today's plants invaded the land sometime during the Paleozoic era (see Table 20.1). The earliest land plants were tiny, but their metabolic activities helped convert native rock into soil that could support the needs of their successors. Larger and larger plants evolved rapidly (in geological terms),

and during the Carboniferous period (354 to 290 million years ago) great forests were widespread. However, few of the trees in those forests were like those we know today. During the tens of millions of years since the Carboniferous, these early trees have been replaced by the modern trees whose adaptations and appearance are familiar to us. In this chapter, we will see how members of the plant kingdom conquered the land and evolved. We will see what made early plants different and made Plantae a unique kingdom, and will survey the diverse products of plant evolution. In the next chapter we will complete our survey of the plant kingdom by considering the seed plants, which dominate the terrestrial scene today.

The Plant Kingdom

As we use the term, a plant is a photosynthetic eukaryote that uses chlorophylls a and b, stores carbohydrates, usually as starch, and develops from an embryo protected by tissues of the parent plant. Most plants have, or had and then lost in the course of evolution, two whiplash flagella at the anterior end of their motile cells. Thus defined, the kingdom Plantae is monophyletic—it forms a single branch of the evolutionary tree. Because of their development from embryos, plants are sometimes referred to as emhryophytes.



r _ • <■

Fuel from Plants

Peat, formed from layers of plant matter including moss of the genus Sphagnum, is the major source of fuel for residents of the Falkland Islands off the coast of Argentina.

>#" Jtf

j\

>



f-

•^'" \ *•

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 501

?\$ t \ Classification of Plants'

PHYLUM

COMMON NAME

CHARACTERISTICS

Hepatophyta

Anthocerophyta

Bryophyta

NONSEED TRACHEOPHYTES

Lycophyta Sphenophyta Psilotophyta Pterophyta

SEED PLANTS

Gymnosperms

Cycadophyta Ginkgophyta Gnetophyta Coniferophyta

Angiosperms

Angiospermae

NONTRACHEOPHYTES

Liverworts No filamentous stage; gametophyte flat

Hornworts Embedded archegonia; sporophyte grows basally

Mosses Filamentous stage; sporophyte grows apically (from the tip)

TRACHEOPHYTES

Club mosses Horsetails Whisk ferns Ferns

Cycads Ginkgo Gnetophytes Conifers

Simple leaves in spirals; sporangia in leaf axils

Simple leaves in whorls; stems jointed

No true leaves; roots absent

Complex leaves; sporangia on underside of leaves

Compound leaves; swimming sperm; seeds on modified leaves Deciduous; fan-shaped leaves; swimming sperm Vessels in vascular tissue; opposite, simple leaves Seeds in cones; needlelike or scalelike leaves

Flowering plants Endosperm; carpels; much reduced gametophytes; seeds in fruit

"No extinct groups are included in this classification.

Some botanists refer to a group consisting of the Plantae plus the green algae as the "green plant kingdom," to the Stramenopila as the "brown plant kingdom," and the Rhodophyta as the "red plant kingdom" (see Figure 27.29).

There are twelve surviving phyla of plants

The surviving members of the kingdom Plantae fall naturally into twelve phyla (Table 28.1). All members of nine of the phyla possess well-developed vascular systems that transport materials throughout the plant body. We call these nine phyla, collectively, the tracheophytes because they all possess conducting cells called tracheids.

The remaining three phyla (liverworts, hornworts, and mosses), which lack tracheids, were once considered classes of a single larger phylum, of which the most familiar examples are mosses. Now we use the term nontracheophytes to refer collectively to these three phyla. The nontracheophytes are sometimes collectively called bryophytes, but in this text we reserve that term for the mosses. Collectively, the nontracheophytes are not a monophyletic group.

Life cycles of plants feature alternation of generations

A universal feature of the life cycles of plants is the alternation of generations (see Figure 9.13b). If we begin looking at the plant life cycle at the single-cell stage—the diploid zygote—then the first phase of the cycle features the formation, by mitosis and cytokinesis, of a multicellular embryo and eventually the mature diploid plant (see Figure 27.22).

This multicellular, diploid plant is the sporophyte ("spore plant"). Cells contained in sporangia (singular sporangium, "spore reservoir") on the sporophyte undergo meiosis to produce haploid, unicellular spores. By mitosis and cytokinesis a spore forms a haploid plant. This multicellular, haploid plant is the gametophyte ("gamete plant") and produces haploid gametes. The fusion of two gametes (syngamy, or fertilization) results in the formation of a diploid cell, the zygote, and the cycle repeats.

The sporophyte generation extends from the zygote through the adult, multicellular, diploid plant; the gametophyte generation extends from the spore through the adult, multicellular, haploid plant to the gamete. The transitions between the phases are accomplished by fertilization and meiosis. In all plants, the sporophyte and gametophyte differ genetically: The former has diploid cells, the latter haploid cells.

The Plantae arose from a green algal lineage

Much evidence indicates that the closest living relatives of the plants are a group of green algae called charophytes. The charophytes, along with some other green algae and the plants, are in a lineage that is sister to the Chlorophyta (see Figure 27.9), but we don't yet know which charophyte lineage is the true sister group to the plants. Stoneworts of the genus *Oiara* are charophytes that resemble plants in terms of their rRNA and DNA sequences, peroxisome contents, mechanics of mitosis and cytokinesis, and chloroplast structure (Figure 28.1f). On the other hand, strong evidence from morphology-based cladistic analysis suggests that the

502 CHAPTER TWENTY-EIGHT



(a) *Cham* sp. (stonewort)

28.1 The Closest Relatives of Land Plants

The plant kingdom probably evolved from a common ancestor shared with the charophytes, a green algal group, (a) Molecular evidence seems to favor stoneworts of the genus *Chara*. (b) Evidence from morphology indicates that the group including this coleochaete alga may be the ancestor of land plants.



(b) *Coleochaete* sp.

sister group of the plants is a group of charophytes that includes the genus *Coleochaete* (Figure 28.1b). *Coleochaete*-like algae have features found in plants, such as plasmodesmata and a tendency to protect the young sporophyte.

Whether more similar to stoneworts or to *Coleochaete*, the ancestral green algae lived at the margins of ponds or marshes, ringing them with a green mat. From these margin habitats, which were sometimes wet and sometimes dry, early plants made the move onto land.

The Conquest of the Land

Plants or their immediate ancestors in the green mat pioneered and modified the terrestrial environment. That environment differs dramatically from the aquatic environment. The most obvious difference is the availability of the water that is essential for life: It is everywhere in the aquatic environment, but hard to find and to retain in the terrestrial environment. Water also provides aquatic organisms with support against gravity; a plant on land, however, must either have some other support system or sprawl unsupported on the ground. A land plant must also use different mechanisms for dispersing its gametes and progeny than its aquatic relatives use. How did terrestrial organisms arise from aquatic ancestors to thrive in such a challenging environment?

Adaptations to life on land distinguish plants from green algae

Most of the characteristics that distinguish plants from green algae are evolutionary adaptations to life on land (Figure 28.2). Many of the characteristics that proved adaptive to land plants probably evolved before the appearance of any of the plant groups we will discuss in this chapter. These characteristics include:

- ▶ The cuticle, a waxy covering that retards desiccation (drying).
- ▶ Gametangia, cases that enclose plant gametes and prevent them from drying. Eggs are housed in archegonia, sperm in antheridia.
- ▶ Embryos, which are young sporophytes contained within a protective structure.
- ▶ Certain pigments that afford protection against the mutagenic ultraviolet radiation that bathes the terrestrial environment.
- ▶ Thick spore walls that prevent desiccation and resist decay.

All these characteristics were probably shared by a plant ancestral to today's plants. Further adaptations to the terrestrial environment appeared as plant evolution continued. We will identify the most important ones in this and the next chapter. For now, let's look at one of the key later adaptations: the appearance of vascular tissues.

Most present-day plants have vascular tissue

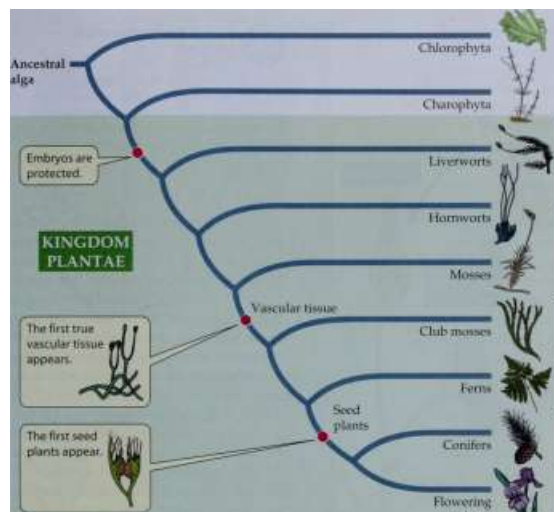
The first plants were truly nonvascular, lacking both water-conducting and food-conducting cells. Although the term "nonvascular plants" is a time-honored name, it is misleading to apply it to the entire nontracheophyte lineage, because some mosses (unlike liverworts and hornworts) do have a limited amount of vascular-like tissue. Thus the more unwieldy name nontracheophyte is more descriptive. The first true tracheophytes—possessing specialized conducting cells called tracheids—arose later.

The nontracheophytes (the liverworts, hornworts, and mosses) have never been large plants. Except for some of the mosses, they have no water-transporting tissue, yet some are found in dry environments. Many grow in dense masses (see Figure 28.7a), through which water can move by capillary action. Nontracheophytes have leaflike structures that readily catch and hold any water that splashes onto them. These plants are small enough that minerals can be distributed internally by diffusion. They lack the leaves, stems, and roots that characterize tracheophytes, although they have structures analogous to each.

Familiar tracheophytes include the ferns, conifers, and flowering plants. Tracheophytes differ from liverworts, hornworts, and mosses in crucial ways, one of which is the possession of a well-developed vascular system consisting of specialized tissues for the transport of materials from one part of the plant to another. One such tissue, the phloem, conducts the products of photosynthesis from sites where they are produced or released to sites where they are used

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 503

Ancestral



"\

- ▶ Nontracheophytes

re re

5

3

- \

Nonseed tracheophytes

re

re

► Seed

plants

Flowering plants

28.2 From Green Algae to Plants

Green algae called charophytes are sister to the plants; green algae called chlorophytes are sister to the lineage that includes the charophytes and plants.

or stored. The other vascular tissue, the xylem, conducts water and minerals from the soil to aerial parts of the plant; because some of its cell walls are stiffened by a substance called lignin, xylem also provides support in the terrestrial environment.

Nontracheophyte plants evolved tens of millions of years before the earliest tracheophytes, even though tracheophytes appear earlier in the fossil record. The oldest tracheophyte fossils date back more than 410 million years, whereas the oldest nontracheophyte fossils are only about 350 million years old, dating from a time when tracheophytes were already widely distributed. This simply means that, given their different structures and the chemical makeup of their cell walls, tracheophytes are more likely to form fossils than nontracheophytes are.

We will examine the adaptations of the tracheophytes later in the chapter, concentrating first on the nontracheophytes.

The Nontracheophytes: Liverworts, Horn worts, and Mosses

Most liverworts, hornworts, and mosses grow in dense mats, usually in moist habitats (see Figure 28.7a). The



Club mosses

Horsetails

Whisk ferns

Ferns

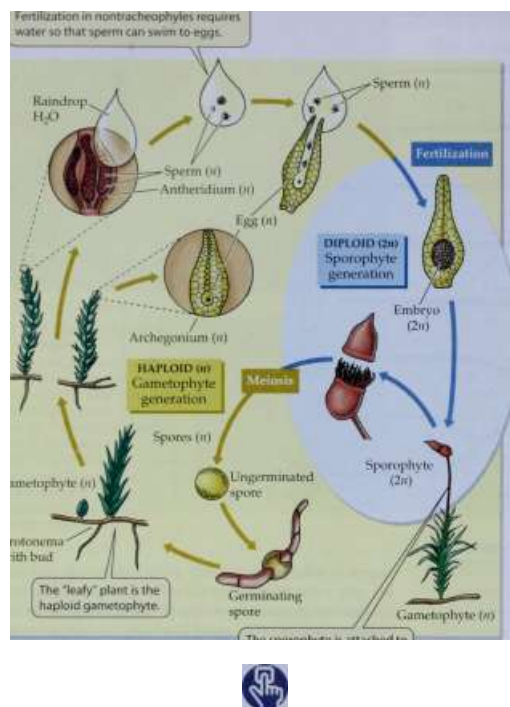
largest of these plants are only about 1 meter tall, and most are only a few centimeters tall or long. Why have no large nontracheophytes ever evolved? The probable answer is that they lack an efficient system for conducting water and minerals from the soil to distant parts of the plant body. However, to limit water loss, layers of maternal tissue protect the embryos of all nontracheophytes. All nontracheophyte lineages also have a cuticle, although it is often very thin (or even absent in

some species) and thus not highly effective in retarding water loss.

Most nontracheophytes live on the soil or on other plants, but some grow on bare rock, dead and fallen tree trunks, and even on buildings. Nontracheophytes are widely distributed over six continents and exist very locally on the coast of the seventh (Antarctica). They are very successful plants, well-adapted to their environments. Most are terrestrial. Some live in wetlands. Although a few nontracheophyte species live in fresh water, these aquatic forms are descended from terrestrial ones. There are no marine nontracheophytes.

504 CHAPTER TWENTY-EIGHT

Fertilization in nontracheophytes requires water so that sperm can swim to eggs.



Gametophyte (/ /)

Protonema with bud

The "leafy" plant is the haploid gametophyte.

Germinating spore

The sporophyte is attached to and nutritionally dependent on the gametophyte.

Nontracheophyte sporophytes are dependent on gametophytes

In nontracheophytes, the conspicuous green structure visible to the naked eye is the gametophyte (Figure 28.3). In contrast, the familiar forms of tracheophytes such as ferns and seed plants are sporophytes. The gametophyte of nontracheophytes is photosynthetic and therefore nutritionally independent, whereas the sporophyte may or may not be photosynthetic but is always dependent on the gametophyte and remains permanently attached to it.

A sporophyte produces unicellular, haploid spores as products of meiosis. A spore germinates, giving rise to a multicellular, haploid gametophyte whose cells contain chloroplasts and are thus photosynthetic. Eventually gametes form within specialized sex organs, the gametangia. The archegonium is a multicellular, flask-shaped female sex organ with a long neck and a swollen base (Figure 28.4a). The base contains a single egg. The antheridium is a male sex organ in which sperm, each bearing two flagella, are produced in large numbers (Figure 28.4b).

Figure 28.3 A Nontracheophyte Life Cycle

The life cycle of nontracheophytes, illustrated here by a moss, is dependent on an external source of liquid water. The visible green structure of nontracheophytes is the gametophyte; in nontracheophyte plants, the "leafy" structures are sporophytes.

Once released, the sperm must swim or be splashed by raindrops to a nearby archegonium on the same or a neighboring plant. The sperm are aided in this task by chemical attractants released by the egg or the archegonium. Before sperm can enter the archegonium, certain cells in the neck of the archegonium must break down, leaving a water-filled canal through which the sperm swim to complete their journey. Note that all of these events require liquid water. On arrival at the egg, one of the sperm nuclei fuses with the egg nucleus to form the zygote. Mitotic divisions of the zygote produce a multicellular, diploid sporophyte embryo. The base of the archegonium grows to protect the embryo during its early growth. Eventually the developing sporophyte elongates sufficiently to break out of the archegonium, but it remains connected to the gametophyte by a "foot" that is embedded in the parent tissue and absorbs water and nutrients from it (see Figure 28.3). The sporophyte remains attached to the gametophyte throughout its life. The sporophyte produces a sporangium, or capsule, within which meiotic divisions produce spores and thus the next gametophyte generation.

The structure and pattern of elongation of the sporophyte differ among the three phyla of nontracheophytes— the liverworts (Hepatophyta), hornworts (Anthocero-phyta), and mosses (Bryophyta). The evolutionary relationships of the three phyla and the tracheophytes can be seen in Figure 28.2.

Liverworts are the most ancient surviving plant lineage

The gametophytes of some liverworts (phylum Hepatophyta) are green, leaflike layers that lie flat on the ground (Figure 28.5a). The simplest liverwort gametophytes, however, are flat plates of cells, a centimeter or so long, that produce antheridia or archegonia on their upper surfaces and water-absorbing filaments called rhizoids on the lower. Liverwort sporophytes are shorter than those of mosses and hornworts, rarely exceeding a few millimeters.

The sporophyte has a stalk that connects capsule and foot. The stalk elongates and thus raises the capsule above

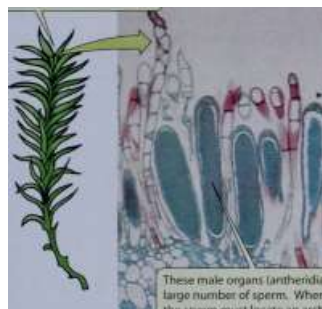
(fl)

Archegonia develop at the tip of a gametophyte. In the archegonium the egg will be fertilized and begin development into a sporophyte.



The large egg cell in the center of the archegonium looks like an eye.

Antheridia are also located at the tip of a gametophyte.



These male organs (antheridia) contain a large number of sperm. When released, the sperm must locate an archegonium and swim down its neck to the egg.

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 505

ground level, favoring dispersal of spores when they are released. The capsules of liverworts are simple: a globular capsule wall surrounding a mass of spores. In some species of liverworts, spores are not released by the sporophyte until the surrounding capsule wall rots.

In other liverworts, however, the spores are disseminated by structures called elaters located within the capsule. Elaters are long cells that have a helical thickening of the cell wall. As an elater loses water, the whole cell shrinks to a fraction of its former length, thus compressing the helical thickening like a spring. When the stress becomes sufficient, the compressed "spring" snaps back to its resting position, throwing spores in all directions.

Among the most familiar liverworts are species of the genus *Marchantia* (Figure 28.5a). *Marchantia* is easily recognized by the characteristic structures on which its male and female gametophytes bear their antheridia and archegonia (Figure 28.5b). Like most liverworts, *Marchantia* also reproduces vegetatively by simple fragmentation of the gametophyte. Along with sexual reproduction, *Marchantia* and some other liverworts and mosses also reproduce vegetatively by means of gemmae (singular gemma), which are lens-shaped clumps of cells. In a few liverworts the gemmae are loosely held in structures called gemma cups, which promote dispersal by raindrops (Figure 28.5c).

Hornworts evolved stomata as an adaptation to terrestrial life

The phylum Anthocerotophyta comprises the hornworts, so named because their sporophytes look like little horns (Figure 28.6). Hornworts appear at first glance to be liverworts with very simple gametophytes. These gametophytes consist of flat plates of cells, a few cells thick.

However, the hornworts, along with the mosses and tracheophytes, share an advance over the liverwort lineage in their adaptation to life on land. They have stomata — pores

28.4 Sex Organs in Plants

Archegonia (a) and antheridia (b) of the moss *Mnium* (phylum Bryophyta). Gametophytes of all plants have archegonia and antheridia, but they are much reduced in seed plants.

28.5 Liverwort Structures

Members of the phylum Hepatophyta display various characteristic structures, (o) Gametophytes. (b) Structures bearing antheridia and archegonia. (c) Gemmae.



(a) *Marchantia* sp.

(b) *Marchantia* sp.

These cups are filled with gemmae—small, lens-shaped outgrowths of the body, each capable of developing into a new plant.

(c) *Lunularia* sp.

506 CHAPTER TWENTY-EIGHT

Anthoceros dieteret

28.6 A Horn wort

The sporophytes of hornworts can resemble little horns.

that, when open, allow the uptake of CO_2 for photosynthesis and the release of O_2 , but that can close to prevent excessive water loss.

Hornworts have two characteristics that distinguish them from both liverworts and mosses. First, the cells of hornworts each contain a single large, platelike chloroplast, whereas the other nontracheophytes contain numerous small, lens-shaped chloroplasts. Second, of all the nontracheophyte sporophytes, those of the hornworts come closest to being capable of indefinite growth (without a set limit).

The stalk of either the liverwort or moss sporophyte stops growing as the capsule matures, so elongation of the sporophyte is strictly limited. In a hornwort such as *Anthoceros*, however, there is no stalk, but a basal region of the capsule remains capable of indefinite cell division, continuously producing new spore-bearing tissue above.

Sporophytes of some hornworts growing in mild and continuously moist conditions can become as tall as 20 centimeters. Eventually the sporophyte's growth is limited by the lack of a transport system. To support their growth, the hornworts need access to nitrogen. Hornworts have internal cavities filled with a mucilage; these cavities are often populated by cyanobacteria that fix atmospheric nitrogen gas into a nutrient form usable by the host plant.

We have presented the hornworts as sister to the lineage consisting of mosses and tracheophytes, but this is only one possible interpretation of the current data. The exact evolutionary status of the hornworts is still in doubt.

Water- and sugar-transport mechanisms emerged in the mosses

The most familiar nontracheophytes are the mosses (phylum Bryophyta). There are more species of mosses than of liverworts and hornworts combined, and these hardy little plants are found in almost every terrestrial environment. They often are found on damp, cool ground, where they form thick mats (Figure 28.7a). The mosses are sister to the tracheophytes (see Figure 28.2).

Many mosses contain a type of cell called a hydroid, which dies and leaves a tiny channel through which water may travel. The hydroid likely is a progenitor of the tracheid, the characteristic water-conducting cell of the tracheophytes, but it lacks lignin (a waterproofing substance) and the wall structure found in tracheids. The possession of hydroids and of a limited system for transport of sucrose by some mosses (via cells called leptoids) shows that the old term "nonvascular plant" is somewhat misleading when applied to mosses.

In contrast to liverworts and hornworts, the sporophytes of mosses and tracheophytes grow by apical cell division. A region at the growing tip provides an organized pattern of cell division, elongation, and differentiation. This allows extensive and sturdy vertical growth of sporophytes.

The moss gametophyte that develops following spore germination is a branched, filamentous structure, or protonema (see Figure 28.3). Although the protonema looks much like a filamentous green alga, it is unique to the mosses. Some of the filaments contain chloroplasts and are photosynthetic; others, called rhizoids, are nonphotosynthetic and anchor the protonema to the substrate. After a period of linear growth, cells close to the tips of the photosynthetic filaments divide

rapidly in three dimensions to form buds. The buds eventually differentiate a distinct tip, or apex, and produce the familiar leafy moss shoot with leaflike structures arranged spirally.

These leafy shoots produce antheridia or archegonia (see Figure 28.4). The antheridia release sperm that travel through liquid water to the archegonia, where they fertilize the eggs. Sporophyte development in most mosses follows a precise pattern, resulting ultimately in the formation of an absorptive foot, a stalk, and, at the tip, a swollen capsule. In contrast to hornworts, which grow from the base, the moss sporophyte stalk grows at its apical end, as tracheophytes do. Cells at the tip of the stalk divide, supporting elongation of the structure and giving rise to the capsule. For a while, archegonial tissue grows rapidly as the stalk elongates, but eventually the archegonium is outgrown and is torn apart by the expanding sporophyte.

The top of the capsule is shed after completing meiosis and spore development. Groups of cells just below the lid form a series of toothlike structures surrounding the opening. Highly responsive to humidity, these structures dig into the mass of spores when the atmosphere is dry; then, when the atmosphere becomes moist, they fling out, scooping out the spores as they go (Figure 28.7b). The spores are thus dispersed when the surrounding air is



28.7 The Mosses

(a) Dense moss forms hummocks in a valley on New Zealand's South Island, (b) The moss capsule, from which spores are dispersed, grows at the tip of the plant.

moist—that is, when conditions favor their subsequent germination.

Only a few mosses depart from this pattern of capsule development. A familiar exception is the genus *Sphagnum*, which we discussed at the beginning of this chapter. Species in this genus have a simple capsule with an air chamber in it. Air pressure builds up in this chamber, eventually causing the capsule lid to pop open, dispersing the spores with an audible explosion.

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 507

With their simple system of internal transport, the mosses are in a sense vascular plants. However, they are not tracheophytes, because they lack true xylem and phloem.

Introducing the Tracheophytes

Although an extraordinarily large and diverse group, the tracheophytes can be said to have been launched by a single evolutionary event. Sometime during the Paleozoic era, probably well before the Silurian period (440 mya), the sporophyte generation of a now long-extinct organism produced a new cell type, the tracheid. The tracheid is the principal water-conducting element of the xylem in all tracheophytes except the angiosperms (flowering plants); and even in angiosperms the tracheid persists alongside a more specialized and efficient system of vessels and fibers derived from tracheids.

The evolutionary appearance of a tissue composed of tracheids had two important consequences. First, it provided a pathway for long-distance transport of water and mineral nutrients from a source of supply to regions of need. Second, it provided something almost completely lacking—and unnecessary—in the largely aquatic green algae: rigid structural support. Support is important in a terrestrial environment because plants tend to grow upward as they compete for sunlight to power

photosynthesis. Thus the tracheid set the stage for the complete and permanent invasion of land by plants.

The tracheophytes feature a further evolutionary novelty: a branching, independent sporophyte. A branching sporophyte can produce more spores than an unbranched body, and it can develop in complex ways. The sporophyte of a tracheophyte is nutritionally independent of the gametophyte.

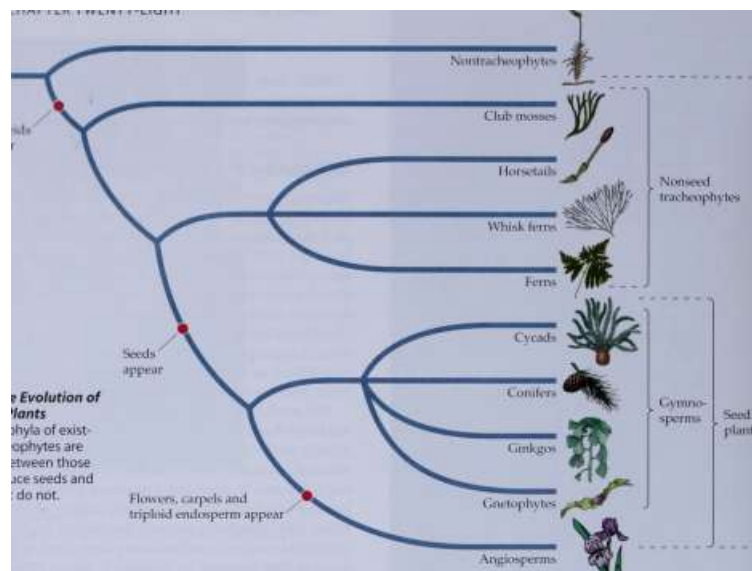
The present-day evolutionary descendants of the early tracheophytes belong to nine distinct phyla (Figure 28.8). We can sort these phyla into two groups: those that produce seeds and those that do not. The nonseed tracheophytes include ferns, horsetails, club mosses, and whisk ferns. In the nonseed tracheophytes, the haploid and diploid generations are independent at maturity. The sporophyte is the large and obvious plant that one normally notices in nature (in contrast to the nontracheophyte sporophyte, which is attached to, dependent on, and usually much smaller than the gametophyte). Gametophytes of the nonseed tracheophytes are rarely more than 1 or 2 centimeters long and are shortlived, whereas their sporophytes are often highly visible; the sporophyte of a tree fern, for example, may be 15 or 20 meters tall and may live for many years.

The most prominent resting stage in the life cycle of a nonseed tracheophyte is the single-celled spore. This feature makes this life cycle similar to those of the fungi, the green algae, and the nontracheophytes but not, as we will see in the next chapter, to that of the seed plants. Nonseed tracheophytes must have an aqueous environment for at least one stage of their life cycle because fertilization is accomplished by a motile, flagellated sperm.

508 CHAPTER TWENTY-EIGHT

Common ancestor

Tracheids appear



Seeds appear

28.8 The Evolution of Today's Plants

The nine phyla of existing tracheophytes are divided between those that produce seeds and those that do not.

Flowers, carpels and triploid endosperm appear

H

Y o -a

We now turn to a more detailed account of the evolution of the nonseed tracheophytes.

Tracheophytes have been evolving for almost half a billion years

The plant kingdom successfully invaded the terrestrial environment between 400 and 500 million years ago. The evolution of a water-impermeable cuticle and of protective layers for the gamete-bearing structures (archegonia and antheridia) helped make the invasion successful, as did the initial absence of herbivores (plant-eating animals).

By the late Silurian period, tracheophytes were being preserved as fossils that we can study today. Several remarkable developments arose during the Devonian period, 409 to 354 million years ago. Three groups of nonseed tracheophytes that still exist made their first appearances during that period: the lycopods (club mosses), horsetails, and ferns. Their proliferation made the terrestrial environment more hospitable to animals: Amphibians and insects arrived soon after the plants became established.

Trees of various kinds appeared in the Devonian period, and dominated the landscape of the Carboniferous. Mighty forests of lycopods up to 40 meters tall, horsetails, and tree ferns flourished in the tropical swamps of what would become North

America and Europe (Figure 28.9). In the subsequent Permian period the continents came ponderously together to form a single gigantic land mass, called Pan-gaea. The continental interior became warmer and drier, but late in the period glaciation was extensive. The 200-million-year reign of the lycopod-fern forests came to an

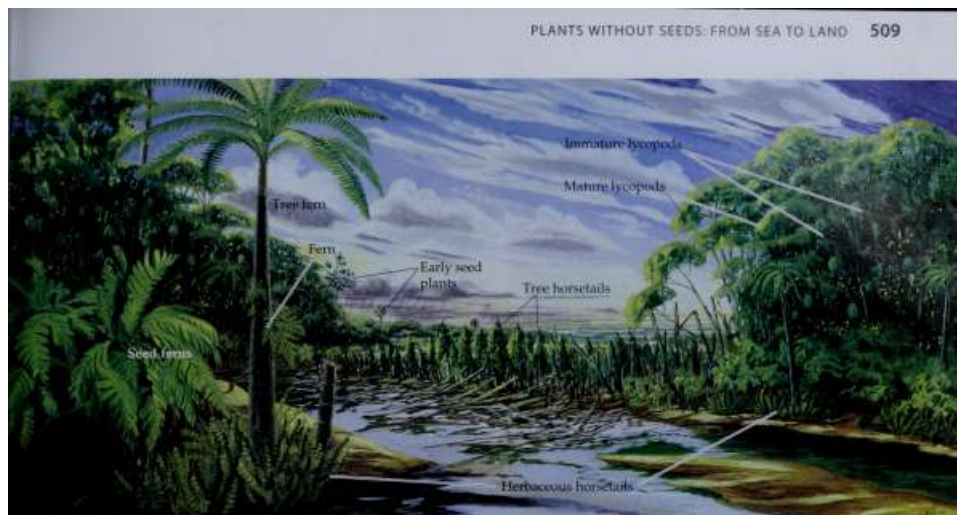
end as they were replaced by forests of seed plants (gym-nosperms) that ruled until other seed plants (angiosperms) became dominant less than 80 million years ago.

The earliest tracheophytes lacked roots and leaves

The first tracheophytes belonged to the now-extinct phylum Rhyniophyta. The rhyniophytes appear to have been the only tracheophytes in the Silurian period. The landscape at that time probably consisted of bare ground, with stands of rhyniophytes in low-lying moist areas. Early versions of the structural features of all the other tracheophyte phyla appeared in the rhyniophytes of that time. These shared features strengthen the case for the origin of all tracheophytes from a common nontracheophyte ancestor.

In 1917, the British paleobotanists Robert Kidston and William H. Lang reported well-preserved fossils of tracheophytes embedded in Devonian rocks near Rhynie, Scotland. The preservation of these plants was remarkable, considering that the rocks were more than 395 million years old. These fossil plants had a simple vascular system of phloem and xylem. Flattened scales on the stems of some of the plants lacked vascular tissue and thus were not comparable with the true leaves of any other tracheophytes.

These plants lacked roots. They were apparently anchored in the soil by horizontal portions of stem, called rhizomes, that bore water-absorbing rhizoids. These rhizomes also bore aerial branches, and sporangia—homologous with the nontracheophyte capsule—were found at



28.9 An Ancient Forest

A little more than 300 million years ago, a forest grew in a setting similar to tropical river delta habitats of today. Most of the plants depicted here were nonseed tracheophytes 10 to 20 m tall. Far in the distance, early seed plants—giants up to 40 m tall—towered over the forest. This artist's impression is based on evidence from fossils.

the tips of these branches. Branching was dichotomous; that is, the shoot apex divided to produce two equivalent new branches, each pair diverging at approximately the same angle from the original stem (Figure 28.10). Scattered fragments of such plants had been found earlier, but never in such profusion or so well preserved as those discovered near Rhynie by Kidston and Lang.

The presence of xylem indicated that these plants, named R_Jn/nia after the site of their discovery, were tracheophytes. But were they sporophytes or gametophytes? Close inspection of thin sections of fossil sporangia revealed that the spores were in groups of four. In almost all living nonseed tracheophytes (with no evidence to the contrary from fossil forms), the four products of a meiotic division and cytokinesis remain attached to one another during their development into spores. The spores separate only when they are mature, and even after separation their walls reveal the exact geometry of how they were attached. Therefore, a group of four closely packed spores is found only immediately after meiosis, and a plant that produces such a group of four must be a diploid sporophyte—and so the Rhynie fossils must have been sporophytes. The gametophytes of the Rhyniophyta also were branched, and depressions at the apices of the branches contained archegonia and antheridia.

Although apparently ancestral to the other tracheophyte phyla, the rhyniophytes themselves are long gone. None of their fossils appear anywhere after the Devonian period.

Dichotomous branching



Sporangia with spores in groups of four determined that fossil structure was sporophyte.

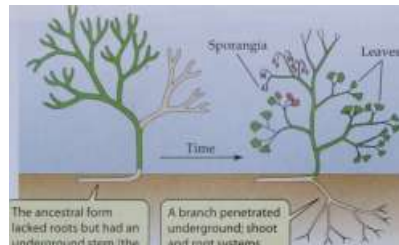
Rhizome

28.70 A Very Ancient Tracheophyte

This extinct plant in the genus *Rhynia* (phylum Rhyniophyta) lacked roots and leaves. The rhizome is a horizontal underground stem, not a root. The aerial shoots were less than 50 cm tall, and some were topped by sporangia.

510 CHAPTER TWENTY-EIGHT

Leaves



The ancestral form lacked roots but had an underground stem (the rhizome) with rhizoids.

A branch penetrated underground; shoot and root systems diverged.

28.11 Is This How Roots Evolved?

According to Lignier's hypothesis, branches from ancestral rootless plants could have penetrated the soil, where they gradually evolved into a root system.

Early tracheophytes added new features

Within a few tens of millions of years, during the Devonian period, three new phyla of tracheophytes—Lycophyta, Sphenophyta, and Pterophyta—appeared on the scene, arising from rhyniophyte-like ancestors. These new groups featured specializations not found in the rhyniophytes, including one or more of the following: true roots, true leaves, and a differentiation between two types of spores.

the origin of roots. Rhynia and its close relatives lacked true roots. They had only rhizoids arising from a rhizome (Figure 28.11, left) with which to gather water and minerals. How, then, did subsequent groups of tracheophytes come to have the complex roots we see today?

In 1903, a French botanist, E. A. O. Lignier, proposed an attractive hypothesis that is still widely accepted today. Lignier argued that the ancestors of the first tracheophytes grew by branching dichotomously. This explanation is supported by the dichotomous branching observed in the rhyniophytes. Lignier suggested that such a branch could bend, penetrate the soil, and branch there (Figure 28.11,

right). The underground portion could anchor the plant firmly, and even in this primitive condition it could absorb water and minerals. The subsequent discovery of fossil plants from the Devonian period, all having horizontal stems (rhizomes) with both underground and aerial branches, supported Lignier's hypothesis.

Underground and aboveground branches, growing in sharply different environments, were subjected to very different selection during the succeeding millions of years. Thus the two parts of the plant axis (the shoot and root systems) diverged in structure and evolved distinct internal and external anatomies. In spite of these differences, scientists believe that the root and shoot systems of tracheophytes are homologous—that they were once part of the same organ.

the origin of true leaves. Thus far we have used the term "leaf" rather loosely. We spoke of "leafy" mosses and commented on the absence of "true leaves" in rhyniophytes. In the strictest sense, a leaf is a flattened photosynthetic structure emerging laterally from a main axis or stem and possessing true vascular tissue. Using this precise definition as we take a closer look at true leaves in the tracheophytes, we see that there are two different types of leaves, very likely of different evolutionary origins.

The first type, the simple leaf, is usually small and only rarely has more than a single vascular strand, at least in plants alive today. Plants in the phylum Lycopphyta (club mosses), of which only a few genera survive, have such leaves. The evolutionary origin of simple leaves is thought by some biologists to be sterile sporangia (Figure 28.12fl). The principal characteristic of this type of leaf is that its vascular strand departs from the vascular system of the stem in such a way that the structure of the stem's vascular system is scarcely disturbed. This was true even in the fossil lycopod trees of the Carboniferous period, many of which had leaves many centimeters long.

28.12 The Evolution of Leaves

{a) Simple leaves are thought to have evolved from sterile sporangia. (5) The complex leaves of ferns and seed plants may have arisen as photosynthetic tissue developed between complex branching patterns.

Vascular tissue

Sporangia

(«)

A sporangium evolved into a simple leaf.

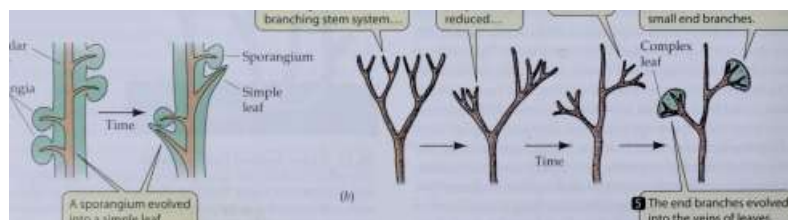
Complex leaves may have originated as a branching stem system...

Q ...becoming progressively reduced...

t

...and flattened.

Q Flat plates of photosynthetic tissue developed between small end branches.



T

The end branches evolved into the veins of leaves.

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 51 1

The other type of leaf is encountered in ferns and seed plants. This larger, more complex leaf is thought to have arisen from the flattening of a dichotomously branching stem system, with the development of extensive photo-synthetic tissue between the branch members (Figure 28.12b). The complex leaf may have evolved several times, in different phyla of tracheophytes.

homospory and heterospory. In the most ancient of the present-day tracheophytes, both the gametophyte and the sporophyte are independent and usually photosynthetic. Spores produced by the sporophytes are of a single type, and they develop into a single type of gametophyte that bears both female and male reproductive organs. Such plants, which bear a single type of spore, are said to be homosporous (Figure 28.13fl). The sex organs on the game-tophytes of homosporous plants are of two types. The female organ is a multicellular archegonium, typically containing a single egg. The male organ is an antheridium, containing many sperm.

A different system, with two distinct types of spores, evolved somewhat later. Plants of this type are said to be heterosporous (Figure 28.13b). One type of spore, the megaspore, develops into a larger, specifically female gametophyte (megagametophyte) that produces only eggs. The other type, the microspore, develops into a smaller, male gametophyte (microgametophyte) that produces only sperm. The sporophyte produces mega-spores in small numbers in megasporangia on the sporophyte, and microspores in large numbers in microsporangia.

The most ancient tracheophytes were all homosporous. Heterospory evidently evolved independently several times in the early evolution of the tracheophytes descended from the rhyniophytes. The fact that heterospory evolved repeatedly suggests that it affords selective advantages. Subsequent evolution in the plant kingdom featured ever greater specialization of the heterosporous condition.

The Surviving Nonseed Tracheophytes

Today ferns are the most abundant and diverse phylum of nonseed tracheophytes, but club mosses and horsetails were once dominant elements of Earth's vegetation. A fourth phylum, the whisk ferns, contains only two genera. In this section we'll look at the characteristics of these four phyla and at some of the evolutionary advances that appeared in them.

The club mosses are sister to the other tracheophytes

The club mosses (lycopods, phylum Lycopphyta) diverged earlier than all other living tracheophytes—that is, the remaining tracheophytes share an ancestor that was not ancestral to the Lycopphyta. There are relatively few surviving species of club mosses. They have roots that branch dichoto-

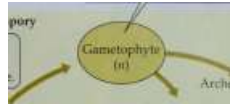
Homosporous plants produce a single type of gametophyte with both male and female reproductive organs.

{a) Homospory

Homosporous

plants produce

a single type of spore.



Spore (n)

/

Meiosis

Gametophyte' (n)

Archegonium (9)

(")

Antheridium (6)

(n)i

Spore mother cell {In)

\

HAPLOID («)

Gametophyte generation

DIPLOID (In) Sporophyte generation

\

Sperm («)

um (x

\

Eggs (h)

J

Fertilization

T

Zygote {In)

Sporangium {2n)

J



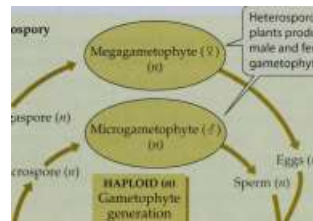
Embryo (2n)

(b) Heterospory

Heterosporous plants produce male and female gametophytes.

Heterosporous plants produce two types of spores: a larger megaspore and a smaller microspore.

Microspore (h)



W- Megaspore (n)

HAPLOID («)

Gametophyte generation

Spore Spore mother mother cell (2n) cell {1n} \

\\

Sporangium (2n)

DIPLOID (2m) Sporophyte generation

Fertilization

Zygote (1n)

J



Embryo (2n)



[rp^ 28.13 Homospory and Heterospory

{a) Homosporous plants bear a single type of spore. Each gametophyte has two types of sex organs, antheridia (male) and archegonia (female). (fc>) Heterospory, with two types of spores that develop into distinctly male and female gametophytes, evolved later.

mously. They bear only simple leaves, and the leaves are arranged spirally on the stem. Growth in club mosses comes entirely from groups of dividing cells at the tips of the stems and thus is apical, as it is in many flowering plants.

512 CHAPTER TWENTY-EIGHT

Leaves

Strobilus

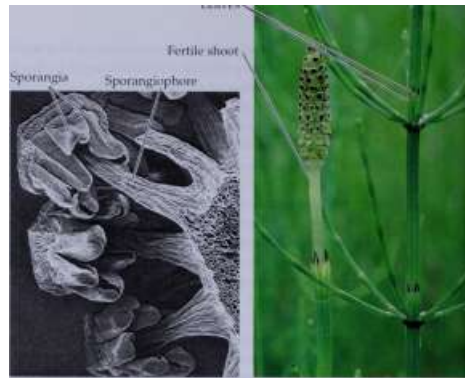


(a) Lycopodium obscurum

28.14 Club Mosses

(a) Strobili are visible at the tips of this club moss. Club mosses have simple leaves arranged spirally on their stems, (b) Thin section through a strobilus. Specialized leaves called sporophylls enclose each sporangium.

Sporangia



(a) Equisetum arvense (b) Equisetum palustre

28.15 Horsetails

(a) Sporangia and sporangiophores of a horsetail, (b) Vegetative and fertile shoots of the marsh horsetail. Leaves form in whorls at nodes on the stems of the vegetative shoot on the right; the fertile shoot on the left is ready to disperse its spores.



Hornworts

Mosses

The sporangia in most club mosses are contained within conelike structures called strobili (singular strobilus; Figure 28.14) and are tucked in the upper angle between a specialized leaf and the stem. This placement contrasts with the terminal sporangia of the rhyniophytes (see Figure 28.10). There are both homosporous species and heterosporous species of club mosses. Like all the nonseed tracheophytes, they have a large, independent sporophyte and a small, independent gametophyte.

Although only a

minor element Liverworts

of present-day vegetation, the Lycophyta are one of two phyla that appear to have been the dominant vegetation during the Carboniferous period. One abundant type of coal (Cannel coal) is formed almost entirely from fossilized spores of a tree lycopod named Lepidodendron —which gives us an

idea of the abundance of this genus in the forests of that time. The other major element of the Carboniferous vegetation was the phylum Sphenophyta, the horsetails.

Horsetails grow at the bases of their segments

Like the club mosses, the horsetails (phylum Sphenophyta) are represented by only a few present-day species. They are sometimes called scouring rushes because silica deposits found in the cell walls made them useful for cleaning. They



Ferns

have true roots that branch irregularly, as do the roots of all tracheophytes except the club mosses. Their sporangia curve back toward the stem on the ends of short stalks (sporangiophores) (Figure 28.15a). Horsetails have a large sporophyte and a small gametophyte, both independent.

The leaves of horsetails are simple and form distinct whorls (circles) around the stem (Figure 28.15fr). Growth in horsetails originates to a large extent from discs of dividing cells just above each whorl of leaves, so each segment of the stem grows from its base. Such basal growth is uncommon in plants, although it is found in the grasses, a major group of flowering plants.

Present-day whisk ferns resemble the most ancient tracheophytes

There once was some disagreement about whether rhyniophytes are entirely extinct. The confusion arose because of the existence today of two genera of rootless, spore-bearing plants, Psilotum and Tmesipteris. Psilotum nudum (Figure 28.16) has only minute scales instead of true leaves, but plants of the genus Tmesipteris have flattened photosynthetic organs with

well-developed vascular tissue. Are these two genera the living relics of the rhyniophytes, or do they have more recent origins?

Psilotum and *Tmesipteris* once were thought to be evolutionary ancient descendants of anatomically simple ancestors. That hypothesis was weakened by an enormous hole in the geologic record between the rhyniophytes, which apparently became extinct more than 300 million years ago, and *Psilotum* and *Tmesipteris*, which are modern plants. DNA sequence data finally settled the question in favor



Psilotum malum

28.16 A Whisk Fern

Aerial branches of a whisk fern, a plant once considered by some to be a surviving rhyniophyte and by others to be a fern. It is now included in the phylum Psilotophyta, and is widespread in the tropics and subtropics.

of a more modern origin from fernlike ancestors. Most botanists now treat these two genera as their own phylum, the Psilotophyta (whisk ferns) rather than as relatives of the rhyniophytes.

We now consider the whisk ferns to be highly specialized plants that evolved fairly recently from anatomically more complex ancestors. Whisk fern gametophytes live below the surface of the ground and lack chlorophyll. They depend upon fungal partners for their nutrition.

Ferns evolved large, complex leaves

The sporophytes of the ferns and seed plants have roots, stems, and leaves. Their leaves are typically large and have branching vascular strands. Some species have small leaves as a result of evolutionary reduction, but even the small leaves have more than one vascular strand.

The true ferns constitute the phylum Pterophyta, which first appeared during the Devonian period and today consists of about 12,000 species. The Pterophyta are probably not a monophyletic group. Ferns are characterized by

Liverworts

Hornworts

Mosses

Club mosses

Horsetails

Whisk ferns

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 513

fronds (large leaves with complex vasculature; Figure 28.17f) and by a requirement for water for the transport of the male gametes to the female gametes. Most ferns inhabit shaded, moist woodlands and swamps. Tree ferns can reach heights of 20 meters. Tree ferns are not as rigid as woody plants, and they have poor root systems. Thus they do not grow in sites exposed directly to

strong winds but rather in ravines or beneath trees in forests. During its development, the fern frond unfurls from a tightly coiled "fiddlehead" (Figure 28.17b). Some fern leaves become climbing organs and may grow to be as much as 30 meters long. The sporangia are found on the undersurfaces of the leaves, sometimes covering the whole undersurface and sometimes only at the edges; in most species the sporangia are clustered in groups called sori (singular, sorus) (Figure 28.18).

The sporophyte generation dominates the fern life cycle

Inside the sporangia, fern cells undergo meiosis to form haploid spores. Once shed, spores travel great distances and eventually germinate to form independent gametophytes. Old World climbing fern, *Lygodium microphyllum*, is currently spreading disastrously through the Florida Everglades, choking off the growth of other plants. This rapid spread is testimony to the effectiveness of wind-borne spores.

28.17 Fern Fronds Take Many Forms

(a) Fronds of maidenhair fern form a pattern in this photograph. (b) The "fiddlehead" (developing frond) of a common

forest fern; this structure will unfurl and expand to give rise to a complex adult frond such as those in (a), (c) The tiny fronds of a water fern.



514 CHAPTER TWENTY-EIGHT



Dryopteris intermedia

28.18 Fern Sori Contain Sporangia

Sori, each with many spore-producing sporangia, form on the underside of a frond of the Midwestern fancy fern.

Fern gametophytes produce antheridia and archegonia, although not necessarily at the same time or on the same gametophyte. Sperm swim through water to archegonia, often on other gametophytes, where they unite with an egg.

The resulting zygote develops into a new sporophyte embryo. The young sporophyte sprouts a root and can thus grow independently of the gametophyte. In the alternating generations of a fern, the gametophyte is small, delicate, and short-lived, but the sporophytes can be very large and can sometimes survive for hundreds of years (Figure 28.19).

Most ferns are homosporous. However, two groups of aquatic ferns, the Marsileales and Salviniaceae, are derived from a common ancestor that evolved heterospory. Mega-spores and microspores of these plants (which germinate to produce female and male gametophytes, respectively) are produced in different sporangia, and the microspores are always much smaller and greater in number than the megaspores.

A few genera of ferns produce a tuberous, fleshy gametophyte instead of the characteristic flattened, photosynthetic structure described earlier. Like the gametophytes of whisk ferns, these tuberous gametophytes depend on a mutualistic fungus for nutrition;* in some genera, even the sporophyte embryo must become associated with the fun-

*In a mutualistic association, both partners—here, the gametophyte and the fungus—profit.

Q When the spores germinate they form small, heart-shaped gametophytes.

Germinating spore \y~, r



QEggs in archegonia on T gametophytes are

fertilized by swimming I sperm from antheridia.

Mature gametophyte

i u ..nc a \ Archeonium

(about 0.5 cm wide) b

/

N.



Spore tetrad

HAPLOID (n)

Gametophyte generation



Antheridium

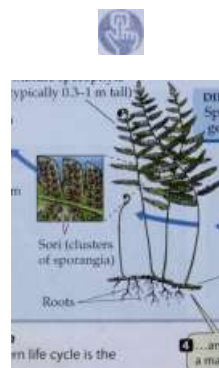
Mature sporophyte (typically 0.3-1 m tall)

Sporangium

Following meiosis, haploid spores emerge from sporangia on the undersides of the sporophyte's fronds.

I 28.19 The Life Cycle of a Fern

The most familiar stage in the fern life cycle is the mature, diploid sporophyte.



DIPLOID (2n)

Sporophyte generation

Sporophyte



Archegonial wall

Embryo -

| The embryo that develops from the diploid zygote in the archegonium sends out roots...

Horizontal stem

.and develops into a mature sporophyte.

PLANTS WITHOUT SEEDS: FROM SEA TO LAND 515

gus before extensive development can proceed. In Chapter 30 we will see that there are many important plant-fungus mutualisms.

All the tracheophytes we have discussed thus far disperse themselves by spores. In the next chapter we discuss the plants that dominate most of Earth's vegetation today, the seed plants, whose seeds afford new sporophytes protection unavailable to the nonseed tracheophytes.

Chapter Summary

- ▶ Plants are photosynthetic eukaryotes that use chlorophylls a and b, store carbohydrates as starch, and develop from embryos protected by parental tissue.
- ▶ Plant life cycles feature alternation of gametophyte (hap-lloid) and sporophyte (diploid) generations.
- ▶ There are twelve surviving phyla of plants grouped into two main categories, nontracheophytes and tracheophytes. Review Table 28.1
- ▶ Plants arose from a common green algal ancestor, either of the stoneworts or of Coleochaete. Descendants of this ancestral charophyte colonized the land.

The Conquest of the Land

- ▶ Moving toward today's plants, early steps in plant evolution included the acquisition of a cuticle, gametangia, a protected embryo, protective pigments, and thick spore walls.
- ▶ Tracheophytes are characterized by possession of a vascular system, consisting of water- and mineral-conducting xylem and nutrient-conducting phloem. Nontracheophytes lack a vascular system. Review Figure 28.2

Nontracheophytes: Liverworts, Hornworts, and Mosses

- ▶ The nontracheophytes include the liverworts (phylum Hepatophyta), hornworts (phylum Anthocerophyta), and mosses (phylum Bryophyta). Review Table 28.1
- ▶ Nontracheophytes either lack vascular tissues completely or, in the case of certain mosses, have only a rudimentary system of water- and food-conducting cells.
- ▶ The nontracheophyte sporophyte generation is smaller than the gametophyte generation and depends on the gametophyte for water and nutrition. Review Figures 28.3, 28.4
- ▶ Liverwort sporophytes have no specific growing zone. Hornwort sporophytes grow at their basal end, and moss sporophytes grow at their apical end. Review Figure 28.6
- ▶ Beginning with hornworts, all plants have surface pores (stomata) that allow gas exchange and minimize water loss.
- ▶ Beginning with mosses, the sporophytes of all plants grow by apical cell division.
- ▶ The hydroids of mosses, through which water may travel, may have arisen from cells also ancestral to the water-conducting cells of the tracheophytes.

Introducing the Tracheophytes

- ▶ The tracheophytes have vascular tissue with tracheids and other specialized cells designed to conduct water, minerals, and foods.
- ▶ Present-day tracheophytes are grouped into nine phyla that form two major groups: nonseed tracheophytes and seed plants. Review Figure 28.8
- ▶ In tracheophytes the sporophyte generation is larger than the gametophyte and independent of the gametophyte generation.
- ▶ The earliest tracheophytes, known to us only in fossil form, lacked roots and leaves. Roots may have evolved from branches that penetrated the ground. Simple leaves are thought to have evolved from sporangia, and complex leaves may have resulted from the flattening and reduction of a branching stem system. Review Figures 28.10, 28.11, 28.12
- ▶ Heterospory, the production of distinct female megaspores and male microspores, evolved on several occasions from homosporous ancestors. Review Figure 28.13

The Surviving Nonseed Tracheophytes

► Club mosses (phylum Lycophyta) have simple leaves arranged spirally. Horsetails (phylum Sphenophyta) have simple leaves in whorls. Whisk ferns (phylum Psilotophyta) lack roots; one genus has minute scales rather than leaves, and the other has leaves with vascular tissue. Leaves with more complex vasculature are characteristic of all other phyla of tracheophytes. Review Table 28.1

► Ferns (phylum Pterophyta) are probably not a mono-phyletic group. They have complex leaves with branching vascular strands. Review Figure 28.19

For Discussion

1. Mosses and ferns share a common trait that makes water droplets a necessity for sexual reproduction. What is this trait?
2. Are the mosses well adapted to terrestrial life? Justify your answer.
3. Ferns display a dominant sporophyte stage (with large fronds). Describe the major advance in anatomy that enables most ferns to grow much larger than mosses.
4. What features distinguish club mosses from horsetails? What features distinguish these groups from rhyniophytes and psilotophytes? From ferns?
5. Why did some botanists once believe that psilotophytes should be classified together with the rhyniophytes?
6. Contrast simple leaves with complex leaves in terms of structure, evolutionary origin, and occurrence among plants.



The Evolution of Seed Plants



A VIOLENT THUNDERSTORM MOVES through forested hills and valleys where summer rain has been scarce. A jagged fork of lightning strikes a tree and it bursts into flame. Soon the flames reach dead and dry underbrush and fire spreads to surrounding trees. The fire rages rapidly through the forest, leaving a blackened and smoking landscape behind.

Though devastating, such fires are a natural part of the forest ecosystem. Life returns quickly following a fire in a natural grassland or forest, in part because some plants have adaptations that enable them to live with fire. One example, obvious from its common name, is fireweed. The seeds of fireweed not only survive fires, but are encouraged by high temperatures to break their dormancy and sprout. Another example is the lodgepole pine tree, which covers vast fire-prone areas in the Rocky Mountains and elsewhere. Its cones will not release their seeds unless the heat of a fire causes them to open.

Seeds are remarkable structures. They protect the plant embryo within them from environmental extremes through what may be a very long resting period. This and other properties contribute to making seed plants the predominant plants on Earth. All of today's forests are dominated by seed plants.

In this chapter we describe the defining characteristics of the seed plants as a group. We survey the diversity of seed plants and describe the flowers and fruits that are characteristic of the flowering plants. Finally, we consider some of the unsolved problems in seed plant evolution.

General Characteristics of the Seed Plants

The most recent group to appear in the evolution of the tracheophytes is the seed plants: the gymno-sperms (such as pines and cycads) and the angio-sperms (flowering plants). There are four living phyla of gymnosperms and one of angiosperms (Figure 29.1). The phylogenetic relationships among these five lineages have not yet been resolved.

A Forest Ablaze

Fires like this one in a northern Arizona forest can pose dangers to human life and property. But they play an essential role in the life cycles of many fire-adapted seed plants.

In seed plants, the gametophyte generation is reduced even further than it is in the ferns (Figure 29.2). The haploid gametophyte develops partly or entirely while attached to and nutritionally dependent on the diploid sporophyte. Among the seed plants, only the earliest types of gymnosperms and their few survivors had swimming sperm. All other seed plants have evolved other means of bringing female and male gametes together. The culmination of this striking evolutionary trend in plants was independence from the liquid water that earlier plants needed for sexual reproduction.

Seed plants are heterosporous, forming separate mega-sporangia and microsporangia on structures that are grouped on short axes, such as the cones of conifers and the flowers of angiosperms.

As in other plants, the spores of seed plants are produced by meiosis within the sporangia, but in seed plants, the megaspores are not shed. Instead, the female gametophytes develop within the megasporangia and depend on them for food and water. In most species only one of the meiotic products in a megasporangium survives. The surviving haploid nucleus divides mitotically, and the resulting cells divide again to produce a multicellular female gametophyte. In the angiosperms, female gametophytes normally contain eight nuclei. The female gametophyte is retained within the megasporangium, where it matures and

/

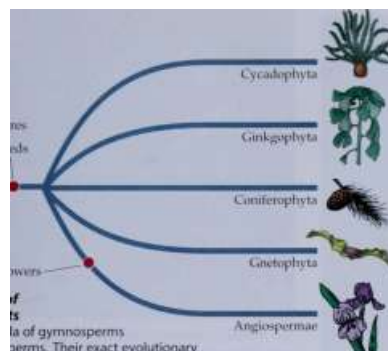


Microspores and megaspores

Seeds

Common ancestor

—•—'



Flowers

29.7 The Phyla of Living Seed Plants

There are four phyla of gymnosperms

and one of angiosperms. Their exact evolutionary

relationship is still uncertain.

houses the early development of the next sporophyte generation following fertilization of the egg. The megasporangium itself is surrounded by sterile sporophyte structures that form a protective integument.

Within the microsporangium, the meiotic products are microspores, which divide within the microspore wall one or a few times to form male gametophytes called pollen grains (Figure 29.3). Distributed by wind, an insect, a bird, or a plant breeder, a pollen grain that reaches the appropriate surface of a sporophyte develops further. It produces a slender pollen tube that elongates and digests its way through the sporophytic tissue toward the female gametophyte.

a ^<

3

3 O

"O rt>

Sporophyte (2n)



Sporophyte (2n)

The moss gametophyte nourishes the sporophyte.

Gametophyte (n)



When the tip of the pollen tube reaches the female gametophyte, two sperm are released from the tube and fertilization occurs. The resulting diploid zygote divides repeatedly, forming a young sporophyte that develops to an embryonic stage at which growth becomes temporarily suspended (often referred to as a dormant stage). The end product at this stage is a seed.

A seed may contain tissues from three generations. The seed coat and megasporangium develop from tissues of the diploid sporophyte parent (the integument). Within the megasporangium is the haploid female gametophyte tissue from the next generation.

- (This tissue is fairly extensive in most

gymnosperm seeds. In angiosperm seeds its place is taken by a tissue called endosperm, which we will discuss shortly.) In the center of the seed package is the third generation, in the form of the embryo of the new diploid sporophyte.

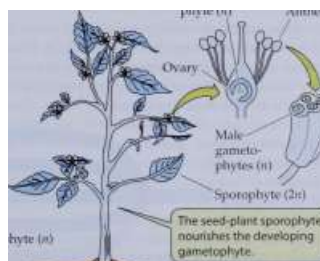
The multicellular seed of a gymnosperm or an angiosperm is a well-protected resting stage. The seeds of some species may remain viable (capable of growth and development) for many years, germinating when conditions are favorable for the growth of the sporophyte. In contrast, the embryos of nonseed plants develop directly into sporophytes, which either survive or die, depending on environmental conditions; there is no resting stage in the life cycle.

29.2 The Relationship between Sporophyte and Gametophyte Has Evolved

In seed plants, the gametophyte (shown in yellow) is nutritionally dependent on the sporophyte (shown in blue).

Female gametophyte (n) Anther

Gametophyte (n)



Sporophyte (2n)

The seed-plant sporophyte nourishes the developing gametophyte.

The large sporophytes and the small gametophytes of ferns are nutritionally independent of one another.





wind carries pollen grains from pollen cone...

a cone.

29.3 Pollen Grains

Pollen grains are the male gametophytes of seed plants. Conifers have separate seed cones (which contain the female gametophyte) and pollen cones; their pollen is dispersed by the wind.

During the dormant stage, the seed coat protects the embryo from excessive drying and may also protect against potential predators that would otherwise eat the embryo and its food reserves. Many seeds have structural adaptations that promote dispersal by wind or, more often, by animals. When the young sporophyte resumes growth, it draws on food reserves in the seed. The possession of seeds is a major reason for the enormous evolutionary success of seed plants, which are the dominant life forms of Earth's modern land flora in most areas.

The Gymnosperms: Naked Seeds

The gymnosperms are a group of seed plants that do not form flowers. Although there are probably fewer than 750 species of living gymnosperms, these plants are second only to the angiosperms (flowering plants) in their dominance of the terrestrial environment.

There are four phyla of living gymnosperms today. The cycads (phylum Cycadophyta) are palmlike plants of the tropics, growing as tall as 20 meters (Figure

29.4f). Ginkgos (phylum Ginkgoeae

Ginkgoeae), which were common during the Mesozoic era, are represented today by a single genus and species, *Ginkgo biloba*, the maidenhair tree (Figure 29.4b). There are both microsporangiate and megasporangiate maidenhair trees. The difference is determined by X and Y sex chromo-



somes, as in humans; few other plants have sex chromosomes. The phylum Gnetophyta consists of three very different genera that share certain characteristics with the angiosperms. One of the gnetophytes is *Welwitschia* (Figure 29.4c), a long-lived desert plant with just two straplike leaves that sprawl on the sand and can become as long as 3 meters. Far and away the most abundant of the gymnosperms are the conifers (phylum Coniferophyta), cone-bearing plants such as pines and redwoods (Figure 29.4d).

All living gymnosperms have stems and roots that grow larger in diameter (called secondary growth), and all but the Gnetophyta have only tracheids as water-conducting and support cells in their xylem. Although the gymnosperm water transport and support system may seem less effective than that of the angiosperms, it serves some of the tallest trees known. The coastal redwoods of California are the tallest gymnosperms; the largest are well over 100 m tall. Secondary xylem—wood—produced by gymnosperms is the principal resource of the timber industry.

Before examining the conifer life cycle, we'll take a brief look at the fossil history of gymnosperms.

We know the early gymnosperms only as fossils

The earliest fossil evidence of gymnosperms is found in Devonian rocks. The early gymnosperms combined characteristics of rhyniophytes and heterosporous ferns, but they had tracheids of the same type found in modern gymnosperms. They also differed from the plants around them by their extensively thickened woody stems, which resulted from proliferation of xylem.

By the Carboniferous period, several new lines of gymnosperms had evolved, including various seed ferns that possessed fernlike foliage but had characteristic gymnosperm seeds attached to their leaves. The first true conifers appeared somewhat later. Either they were not dominant trees or they did not grow where conditions were right for fossilization, so we have few

preserved examples. During the Permian period, however, the conifers and cycads flourished. Gymnosperm forests changed with time as the gymnosperm groups evolved, and they dominated the Mesozoic era, in which the continents drifted apart and dinosaurs strode the Earth. Gymnosperms dominated all forests until less than 100 million years ago, and they still dominate some present-day forests.

Conifers have cones but no motile cells

The great Douglas fir and cedar forests of the northwestern United States and the massive boreal forests of pine, fir, and spruce that clothe the northern continental regions and upper slopes of mountain ranges rank among the great vegetation formations of the world. All these trees belong to one phylum of gymnosperms, Coniferophyta—the conifers, or cone-bearers. A cone is an axis bearing a tight cluster of scales or leaves specialized for reproduction. Megaspores and microspores are produced in separate seed and pollen cones. Seed cones are much larger than pollen cones (see Figure 29.3).

v ft

THE EVOLUTION OF SEED PLANTS 519

^%mr

; -*\

(a) *Cycas* sp.



{b) *Ginkgo biloba*



(c) *Welwitschia mirabilis*

29.4 Diversity among the Gymnosperms

(a) This palm belongs to the cycads, the least changed group of present-day gymnosperms. Many cycads have growth forms that resemble both ferns and palms, (b) The characteristic fleshy seed coat and broad leaves of the maidenhair tree, (c) A gnetophyte growing in the Namib Desert of Africa. Two huge, straplike leaves grow throughout the life of the plant, breaking and splitting as they grow, (d) A dramatic conifer, this giant sequoia grows in Yosemite National Park, California.



(d) *Sequoiadendron giganteum*

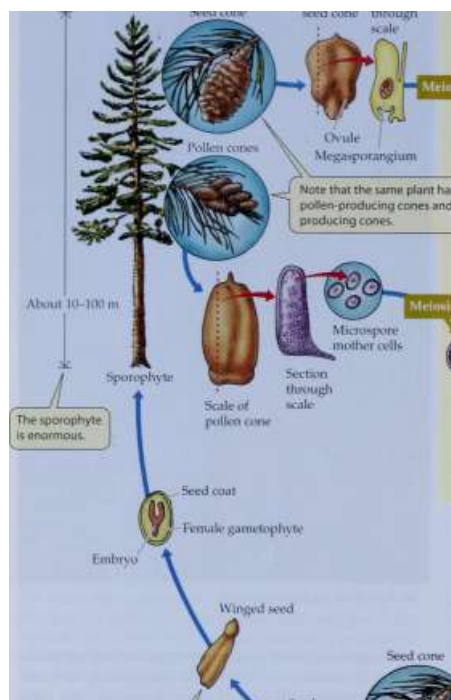
We will use the life cycle of a pine to illustrate reproduction in gymnosperms (Figure 29.5). The production of male gametophytes in the form of pollen grains frees the plant completely from its dependence on liquid water for fertilization. Instead of water, wind assists conifer pollen grains in their first stage of travel to the female gametophyte inside the seed cone. The pollen tube provides the means for the last stage of travel by elongating and digesting its way through maternal sporophytic tissue. When it reaches the female gametophyte, it releases two sperm, one of which degenerates after the other unites with the egg.

The megasporangium, which will form the female gametophyte containing eggs within archegonia, is enclosed in a layer of sporophytic tissue—the integument—that will eventually develop into the seed coat. The integument, the megasporangium inside it, and the tissue attaching it to the maternal sporophyte constitute the ovule. The pollen grain enters through a small opening in the integument at the tip of the ovule, the micropyle.

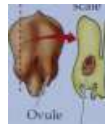
Gymnosperms derive their name (which means "naked-seeded") from the fact that their ovules and seeds are not protected by flower or fruit tissue. Most conifer ovules (which upon fertilization develop into seeds) are borne ex-

520 CHAPTER TWENTY-NINE

Seed cone



Scale of Section seed cone through scale



Functional megaspore

7

Ovule

Megasporangium

Note that the same plant has both pollen-producing cones and egg-producing cones.

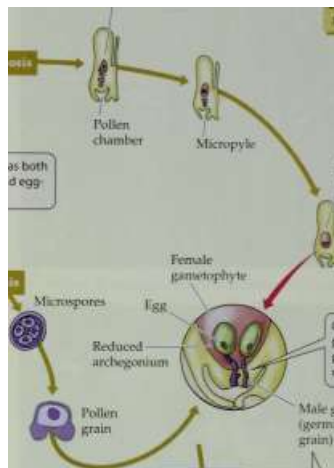
Section through

Scale of scale

pollen cone

Seed coat

Female gametophyte



HAPLOID

Gametophyte

generation

Female gametophyte

Germinating pollen produces pollen tubes to reach the egg.

Male gametophyte (germinating pollen grain)

_f\

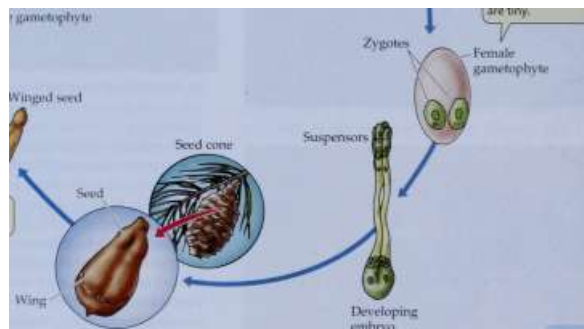
Fertilization

The gametophytes are tiny.

Embryo

Winged seed

The seed coat protects the embryo.



Scale of seed cone

Developing embryo

DIPLOID

Sporophyte generation

In



29.5 The Life Cycle of a Pine Tree

The gametophytes are microscopically small and nutritionally dependent on the sporophyte generation.

posed on the upper surfaces of modified branches called cone scales. Their only protection from the environment lies in the fact that the scales are tightly pressed against each other within the cone. As we have seen, some pines, such as

the lodgepole pine, have such tightly closed seed cones that only fire suffices to split them open and release the seeds.

About half of the conifer species have soft, fleshy fruitlike tissues associated with their seeds; examples are the "berries" of juniper and yew. Animals may eat these tissues and then disperse the seeds in their feces, often carrying them considerable distances from the parent plant. These

tissues, however, are not true fruits, which are characteristic of the plant phylum that is dominant today: the angiosperms.

The Angiosperms: Flowering Plants

The phylum Angiospermae consists of the flowering plants, also commonly known as the angiosperms. This highly diverse phylum includes more than 230,000 species. The oldest evidence of angiosperms dates to the late Jurassic period, more than 140 mya. The angiosperms radiated explosively and, over a period of only about 60 million years, became the dominant plant life of the planet. In later chapters, when we mention "plants," we are generally referring to the angiosperms.

The angiosperms represent the current extreme of an evolutionary trend that runs throughout the tracheophytes: The sporophyte generation becomes larger and more independent of the gametophyte, while the gametophyte generation becomes smaller and more dependent on the sporophyte.

Angiosperms differ from other plants in several ways:

- ▶ They have double fertilization.
- ▶ They produce a diploid endosperm.
- ▶ Their ovules and seeds are enclosed in a carpel.
- ▶ They have flowers.
- ▶ They produce fruit.
- ▶ Their xylem contains vessel elements and fibers.
- ▶ Their phloem contains companion cells.

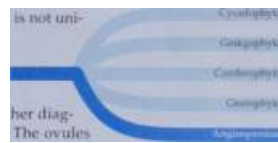
Double fertilization was long considered the single most reliable distinguishing characteristic of the angiosperms. Two male gametes, contained within a single microgametophyte (pollen grain), participate in fertilization events within the megagametophyte of an angiosperm. One sperm combines with the egg to produce a diploid zygote, the first cell of the sporophyte generation. In most angiosperms, the other sperm nucleus combines with two other haploid nuclei of the female gametophyte to form a triploid ($3n$) nucleus. This nucleus, in turn, divides to form a triploid tissue, the endosperm, that nourishes the embryonic sporophyte during its early development.

Double fertilization occurs in all present-day angiosperms. We are not sure when and how it evolved because there is no

fossil evidence on this point. It probably first resulted in two embryos, as it does in the three existing genera of Gnetales: *Ephedra*, *Gnetum*, and *Welwitschia*. Both of the fertilizations in gnetales produce diploid products.

The formation of an extensive triploid endosperm is one of the

The pistil receives pollen, r



most definitive angiosperm traits, although it is not universal.

The name angiosperm ("enclosed seed") is drawn from another diagnostic character: The ovules and seeds of these plants are enclosed in a modified leaf called a carpel. Besides protecting the ovules and seeds, the carpel often interacts with incoming pollen to prevent self-pollination, thus favoring cross-pollination and increasing genetic diversity. Of course, the most evident diagnostic feature of angiosperms is that they have flowers. Production of a fruit is another unique characteristic of the angiosperms.

Angiosperms are also distinguished by the possession of specialized water-transporting cells called vessel elements in their xylem, but these cells are also found, in anatomically different form, in gymnosperms and a few ferns. A second distinctive cell type in angiosperm xylem is the fiber, which plays an important role in supporting the plant body. Angiosperm phloem possesses another unique cell type, called a companion cell.

In the following sections we'll examine the structure and function of flowers, evolutionary trends in flower structure, the functions of pollen and fruits, the angiosperm life cycle, the two major groups of angiosperms, and the origin and evolution of flowering plants.

The sexual structures of angiosperms are flowers

If you examine any familiar flower, you will notice that the outer parts look somewhat like leaves. In fact, all the parts of a flower are modified leaves.

A generalized flower (for which there is no exact counterpart in nature) is shown in Figure 29.6 for the purpose of identifying its parts. The structures bearing microsporangia are called stamens. Each stamen is composed of a filament bearing an anther that contains pollen-producing microsporangia. The structures bearing megasporangia are the carpels. A structure composed of one carpel or two or more fused carpels is called a pistil. The swollen base of the pistil, containing one or more ovules (each containing a

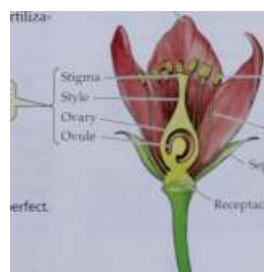
Petal



[m^ 29.6 A Generalized Flower

Not all flowers possess all the structures shown here, but they must possess a stamen (male), pistil (female), or both in order to play their role in reproduction.

Flowers that have both, as this one does, are referred to as perfect.



Anther (micro-sporangium)

Filament



The stamen produces pollen.

Sepal



(a) *Daucus carota*

Compound umbel

29.7 Inflorescences

(a) The inflorescence of Queen Anne's lace is an umbel. Each umbel bears flowers on stalks that arise from a common center. (b) Cornflowers are members of the aster family; their inflorescence is a head. In a head, each of the long, petal-like structures is a ray flower; the central portion of the head consists of dozens to hundreds of disc flowers. (c) Grasses such as this fountain grass have inflorescences called spikes.

megasporangium), is called the ovary. The apical stalk of the pistil is the style; and the terminal surface that receives pollen grains is called the stigma.

In addition, a flower often has several specialized sterile (non-spore-bearing) leaves: The inner ones are called petals (collectively, the corolla), and the outer ones sepals (collectively, the calyx). The corolla and calyx, which can be quite showy, often play roles in attracting animal pollinators to the flower. The calyx more commonly protects the immature flower in bud. From base to apex, the sepals, petals, stamens, and carpels (which are referred to as the floral organs; see Figure 15.11) are usually in circular arrangements called whorls and attached to a central stalk called the receptacle.

The generalized flower shown in Figure 29.6 has both megasporangia and microsporangia; such flowers are referred to as perfect. Many angiosperms produce two types of flowers, one with only megasporangia and the other with only microsporangia. Consequently, either the stamens or the carpels are nonfunctional or absent in a given flower, and the flower is referred to as imperfect.

Species such as corn or birch, in which both megasporangiate and microsporangiate flowers occur on the same plant, are said to be monoecious (meaning "one-housed"—but, it must be added, one house with separate rooms). Complete separation is the rule in some other angiosperm species, such as willows and date palms; in these species, a given plant produces either flowers with stamens or flowers with pistils, but never both. Such species are said to be dioecious ("two-housed").

Flowers come in an astonishing variety of forms, as you will realize if you think of some of the flowers you recognize. The generalized flower shown in Figure 29.6 has distinct petals and sepals arranged in distinct whorls. In

(c) *Pennisetum setaceum*

nature, however, petals and sepals sometimes are indistinguishable. Such appendages are called tepals. In other flowers, petals, sepals, or tepals are completely absent.

Flowers may be single, or grouped together to form an inflorescence. Different families of flowering plants have their own, characteristic types of inflorescences, such as the umbels of the carrot family, the heads of the aster family, and the spikes of many grasses (Figure 29.7).

Flower structure has evolved over time

The flowers that are evolutionarily the most ancient have a large and variable number of tepals (or sepals and petals), carpels, and stamens (Figure 29.8a). Evolutionary change within the angiosperms has included some striking modifications from this early condition: reduction in the number of each type of organ to a fixed number; differentiation of petals from sepals; and change in symmetry from radial (as in a lily or magnolia) to bilateral (as in a sweet pea or orchid), often accompanied by an extensive fusion of parts (Figure 29.8b).

According to one theory, the first carpels to evolve were modified simple leaves, folded but incompletely closed, and thus differing from the scales of the gymnosperms. In the groups of angiosperms that evolved later, the carpels fused and became progressively more buried in receptacle tissue (Figure 29.9a). In the flowers of the latest groups to evolve, the other flower parts are attached at the very top of the ovary, rather than at the bottom as in Figure 29.6. The stamens of the most ancient flowers may have appeared leaflike (Figure 29.9b), little resembling those of the generalized flower in Figure 29.6.

Why do so many flowers have pistils with long styles and anthers with long filaments? Natural selection has favored length in both of these structures, probably because length increases the likelihood of successful pollination. Long filaments may bring the anthers into contact with insect bodies, or they may place the anthers in a better position to catch the wind. Similar arguments apply to long styles.

THE EVOLUTION OF SEED PLANTS 523



(a) Carpel evolution

(a)

29.8 Flower Form and Evolution

{a) A magnolia flower shows the major features of early flowers: It is radially symmetrical, and the individual tepals, carpels, and stamens are separate, numerous, and attached at their bases, {b) Orchids have a bilaterally symmetrical structure that evolved much later than the form of the magnolia flower in (a). One of the three petals evolved into the complex lower "lip." Inside, the stamen and pistil are fused, and there is a single anther in this species.

A long style may serve another purpose as well. If several pollen grains land on one stigma, a pollen tube will start growing from each grain toward the ovary. If there are more pollen grains than ovules, there is a "race" to fertilize the ovules. The race down the style can be viewed as "mate selection" by the plant bearing that style.

Angiosperms have coevolved with animals

Pollen has played another crucial role in the evolution of the angiosperms. Whereas many gymnosperms are wind-pollinated, most angiosperms are animal-pollinated. Animals visit flowers to obtain nectar or pollen, and in the process often carry pollen from one flower to another, or from one plant to another. Thus, in its quest for food, the animal contributes to the genetic diversity of the plant population. Insects, especially bees, are among the most important pollinators; birds and some species of bats also play major roles.

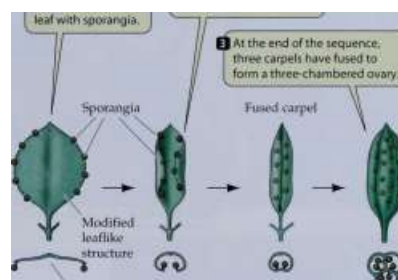
For more than 130 million years, angiosperms and their animal pollinators have coevolved in the terrestrial environment. The animals have affected the evolution of the plants, and the plants have affected the evolution of the animals. Flower structure has become incredibly diverse under these selection pressures.

Q According to one theory, the carpel began as a modified leaf with sporangia.

Q In the course of evolution, leaf edges curled inward and finally fused.

)

§J At the end of the sequence, three carpels have fused to form a three-chambered ovary.



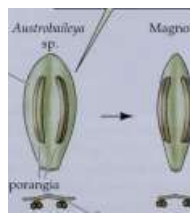
Cross section

(b) Stamen evolution

I]The leaflike portion of the structure was progressively reduced...

Magnolia

Modified leaf



I ...until only the microsporangia remained.



Sporangia

Cross section

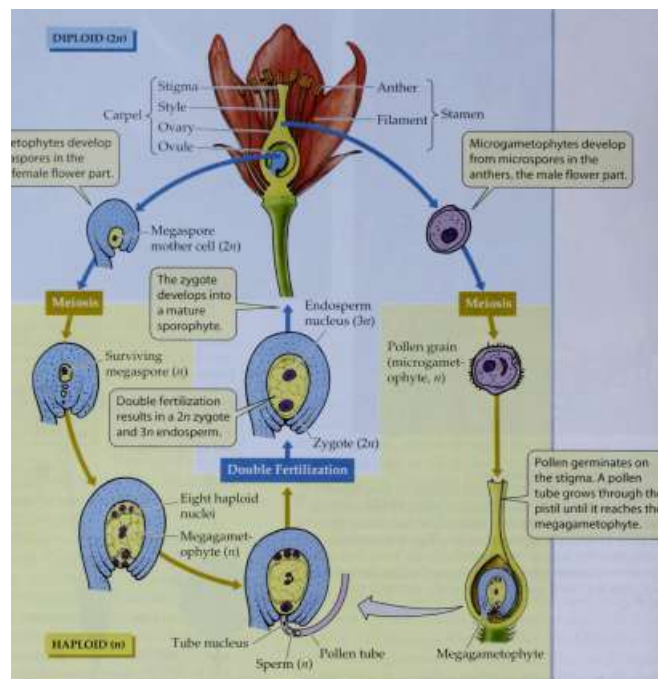
29.9 Carpels and Stamens Evolved from Leaflike Structures

(a) Possible stages in the evolution of a carpel from a more leaflike structure, (b) The stamens of three modern plants show the various stages in the evolution of that organ.

524 CHAPTER TWENTY-NINE

DIPLOID (2n)

Megagametophytes develop from megaspores in the ovule, the female flower part.



29.10 The Life Cycle of an Angiosperm

The formation of a triploid endosperm distinguishes the angiosperms from the gymnosperms.

Some of the products of coevolution are highly specific; for example, some yucca species are pollinated by only one species of moth. Pollination by just one or a very few animal species provides a plant species with a reliable mechanism for transferring pollen from one to another of its members.

Most plant-pollinator interactions are much less specific; that is, many different animal species pollinate the same plant species, and the same animal species pollinate many plant species. However, even these less specific interactions have developed some specialization. Bird-pollinated flowers are often red and odorless. Insect-pollinated flowers often have characteristic odors, and bee-pollinated flowers may have conspicuous markings, or nectar guides, that are evident only in the ultraviolet region of the spectrum, where bees have better vision than in the red region. Co-

evolution and other aspects of plant-animal interactions are covered in more detail in Chapter 55.

The angiosperm life cycle features double fertilization

The life cycle of the angiosperms is summarized in Figure 29.10. The angiosperm life cycle will be considered in detail in Chapter 38, but let's look at it briefly here and compare it with the conifer life cycle in Figure 29.5.

Like all seed plants, angiosperms are heterosporous. The female gametophyte is even more reduced than that of the gymnosperms. The ovules are contained within carpels, rather than being exposed on the surfaces of scales, as in most gymnosperms. The male gametophytes are, again, pollen grains.

The ovule develops into a seed containing the products of the double fertilization that characterizes angiosperms. The triploid endosperm serves as storage tissue for starch or lipids, proteins, and other substances that will be needed by the developing embryo.



(«)

29.11 Fleshy Fruits Come in Many Forms and Flavors

(a) A simple fruit (sour cherries), (fc>) An aggregate fruit (raspberries), (c) A multiple fruit (pineapple), (d) An accessory fruit (pear).

The diploid zygote develops into an embryo, consisting of an embryonic axis and one or two cotyledons. Also called seed leaves, the cotyledons have different fates in different plants. In many, they serve as absorptive organs that take up and digest the endosperm. In others, they enlarge and become photosynthetic when the seed germinates. Often they play both roles (see Chapter 37).

Angiosperms produce fruits

The ovary of a flowering plant (together with the seeds it contains) develops into a fruit after fertilization. A fruit may consist only of the mature ovary and its seeds, or it may include other parts of the flower or structures associated with it. A simple fruit, such as a cherry (Figure 29.11rt), is one that develops from a single carpel or several united carpels. A raspberry is an example of an aggregate fruit (Figure 29.11b)—one that develops from several separate carpels of a single flower. Pineapples and figs are examples of multiple fruits (Figure 29.11c), formed from a cluster of flowers (an inflorescence). Fruits derived from parts in addition to the carpel and seeds are called accessory fruits (Figure 29.11d); examples are apples, pears, and strawberries. The development, ripening, and dispersal of fruits will be considered in Chapters 37 and 38.

Determining the oldest living angiosperm lineage

Which angiosperms were the first flowering plants was long a matter of great controversy. Two leading candidates were the magnolia family (see Figure 29.8(7)) and another family, the Chloranthaceae, whose flowers are much simpler than those of the magnolias. At the close of the twentieth century, an impressive convergence of evidence led to the conclusion that the base of the angiosperm phylogenetic tree belongs to neither of those families, but rather to a lineage that today consists of just a single species of the



(b)



(c)



(d)

genus *Amborella* (Figure 29.12). This woody shrub, with cream-colored flowers, lives only on New Caledonia, an island in the South Pacific. Its 5 to 8 carpels are in a single whorl, and it has 30 to 100 stamens. The xylem of *Amborella* lacks vessel elements, which appeared later in angiosperm evolution. The characteristics of *Amborella* give us a good sense of what the first angiosperms might have been like.

526 CHAPTER TWENTY-NINE



29.12 The First Angiosperm

29.12 The First Angiosperm

(a) *Amborella*, a shrub, is the closest living relative of the first angio-sperms. (b) A flower of *Amborella*.

(b)

There are two large monophyletic groups of angiosperms

There are two large lineages that include the great majority of angiosperm species: the monocots and the eudicots. Both are monophyletic groups (Figure 29.13). The monocots are so called because they have a single embryonic cotyledon; the eudicots have two. The cotyledons of some, but not all, eudicots store the reserves originally present in the endosperm. There are several other differences between the two lineages, which we will describe in Chapter 34. Some familiar plants, including magnolias and water lilies, belong to lineages more ancient than either the monocots or the eudicots.

The monocots (Figure 29.14) include grasses, cattails, lilies, orchids, and palm trees. The eudicots (Figure 29.15) include the vast number of familiar seed plants, including most of the herbs, vines, frees,

and shrubs. Among them are oaks, willows, violets, snapdragons, and sunflowers.

The origin of the angiosperms remains a mystery

We have learned a lot about evolution within the angiosperm lineage. The most important unanswered question about the evolution of seed plants is this: How did the angiosperms first arise—to which gymnosperm phylum are they sister? You might think that, given the advances in techniques of molecular genetics and computer technology, as well as new fossil finds,

we would have opened this century with the answer to this question well in hand. A very few years ago, it seemed that we were on the verge of answering it. Although an answer may be agreed upon before the present decade ends, the puzzle is as vexing today as it was before.

Why should this be? Different phylogenetic methods, applied by different investigators, have produced apparently contradictory results. It might seem a simple matter to rectify this situation, but several questions complicate such efforts: What morphological characters should be selected as important, or should they all be treated as equally important? What algorithms should be applied to computerized analysis of data? Are all molecular differences and similarities significant, or are some of them incidental? Which fossils should be chosen for comparisons? What is the likelihood that we can find evidence of double fertilization in ancient fossils?

We are left with the question: Where did the first angiosperm come from? The angiosperms may be most closely related to the gnetophytes, or they may be more closely related to the conifers, or to another gymnosperm phylum. Current progress in methodology gives us reason to hope that our picture of seed plant evolution will be much more complete before this decade ends. We will see in Chapters 31-33 whether our understanding of animal evolution is more complete.

Carpels; triploid endosperm; seeds in fruit

Gymno-

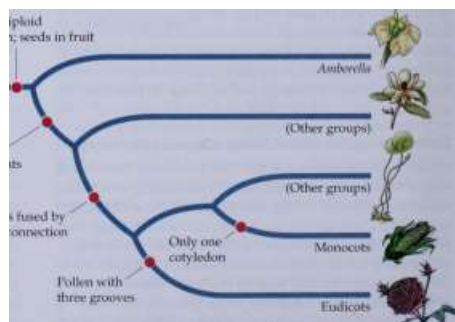
sperm-like

ancestor

Vessel elements

29.13 Evolutionary Relationships among the Angiosperms

The monocots and the eudicots are the largest monophyletic groups among the angiosperms. A few lineages that differ from the monocots, eudicots, and Amborella remain to be placed accurately on the phylogenetic tree.



Carpels fused by tissue connection

Pollen with three grooves

V. wf

THE EVOLUTION OF SEED PLANTS



29.74 Monocots

(a) Palms are among the few monocot trees. Date palms are a major food source in some areas of the world, (b) Grasses such

as this cultivated wheat and the fountain grass in Figure 29.7c are monocots. (c) Monocots include popular garden flowers such as these daylilies. Orchids (Figure 29.85) are another highly prized monocot flower.

(c) *Hemerocallis* sp.



(c) *Rosa rugosa*

29.15 Eudicots

(a) The cactus family is a large group of eudicots, with about 1,500 species in the Americas. This cactus bears scarlet flowers for a brief period of the year, (b) The flowering dogwood is a small eudicot tree, (c) Climbing Cape Cod roses are members of the eudicot family Rosaceae, as are the familiar roses from your local florist.

528 CHAPTER TWENTY-NINE

Chapter Summary

General Characteristics of the Seed Plants

- The seed plants (gymnosperms and angiosperms) are heterosporous and have greatly reduced gametophytes. Review Figures 29.1, 29.2
- Most modern seed plants have no swimming gametes and do not require liquid water for fertilization. The male gametophyte—the pollen grain—is dispersed by wind or by animals. Review Figure 29.3
- The seed is a well-protected resting stage that often contains food that supports the growth of the embryo.

The Gymnosperms: Naked Seeds

- The gymnosperms, once the dominant vegetation on Earth, still dominate forests in the northern parts of the Northern Hemisphere and at high elevations.
- The four surviving gymnosperm phyla are the Cycadophyta (the most ancient), Ginkgophyta (consisting of a single species, the maidenhair tree), Gnetophyta (which has some characters in common with the angiosperms), and Coniferophyta (the familiar cone-bearing trees).
- Modern gymnosperms all have abundant xylem and extensive secondary growth.
- Conifers have a life cycle in which naked seeds are produced on the scales of female cones. Pollen cones are smaller than seed cones. Pollen is transferred from pollen cones to seed cones by wind. Review Figure 29.5

The Angiosperms: Flowering Plants

- Angiosperms (phylum Angiospermae) are distinguished by double fertilization, which results in a triploid nutritive tissue, the endosperm. Double fertilization is also characteristic of the Gnetophyta. Review Figure 29.10
- The ovules and seeds of angiosperms are enclosed by a carpel. Angiosperms are also characterized by the production of flowers and fruits.
- The vascular tissues of angiosperms contain three characteristic cell types: vessel elements, fibers, and companion cells.
- Flowers are made up of various combinations of carpels, stamens, petals, and sepals. Perfect flowers have both carpels (female parts) and stamens (male parts). Review Figure 29.6
- Monoecious plant species have both female and male flowers on the same plant. Dioecious species have separate female and male plants.

- ▶ Carpels and stamens may have evolved from leaflike structures. Review Figure 29.9
- ▶ Angiosperms and the animals that pollinate them have coevolved.
- ▶ Amborella, a tropical shrub, is the sole living representative of the first angiosperm lineage.
- ▶ There are two major lineages of flowering plants: monocots and eudicots. Review Figure 29.13
- ▶ The evolutionary origin of the angiosperms remains a mystery.

For Discussion

1. In most seed plant species, only one of the products of meiosis in the megasporangium survives. How might this be advantageous?
2. Suggest an explanation for the great success of the angiosperms in occupying terrestrial habitats.
3. In many locales, large gymnosperms predominate over large angiosperms. Under what conditions might gymnosperms have the advantage, and why?
4. Not all flowers possess all of the following parts: sepals, petals, stamens, and carpels. What kind or kinds of flower parts do you think might be found in the flowers that have the smallest number of kinds? Discuss the possibilities, both for a single flower and for a species.
5. The problem of the origin of the angiosperms has long been "an abominable mystery," as Charles Darwin once put it. Scientists still do not know the nearest relatives of the angiosperms. It has often been suggested (correctly or incorrectly) that the gnetophytes are sister to the angiosperms. What pieces of evidence suggested this connection?



Fungi: Recyclers, Killers, and Plant Partners



What are the largest organisms you can think of? Whales? Trees? Some of the largest organisms on Earth are fungi. One such fungus, growing in Michigan, covers an area of 37 acres. Its effect on green plants is evident from the air, but from ground level, it is difficult to realize how large the fungus is. At the surface, you see only seemingly isolated clumps of mushrooms. But the vast body of the fungus *Armillariella*, which weighs approximately the same as a blue whale, grows underground and consists almost entirely of microscopic filaments.

Molecular studies indicate that this giant fungus is or was a single individual that arose from a single spore. It is possible that fragmentation over time may have broken it into a few separate—but still gigantic—individuals. Another, larger fungus of the same genus, growing in the state of Washington, occupies parts of three counties. But not all fungi are huge. Molds and mushrooms are fungi, as are the microscopic, unicellular yeasts.

Every breath we take contains large numbers of fungal spores. Some of those spores can be dangerous, and fungal diseases of humans, some of which are as yet incurable, have become a major global threat. However, other fungi are of immense commercial importance to us. Fungi are essential to plants as well. Fungi interact with roots, greatly enhancing the roots' ability to take up water and mineral nutrients.

Earth would be a messy place without the fungi. They are at work in forests, fields, and garbage dumps, breaking down the remains of dead organisms (and even manufactured substances such as some plastics). For almost a billion years, the ability of fungi to decompose substances has been important for life on Earth, chiefly because by breaking down carbon compounds, they return carbon and other elements to the environment, where they can be used again by other organisms.

In this chapter we will examine the general biology of the kingdom Fungi, which differs in interesting ways from the other kingdoms. We will also explore the diversity of body forms, reproductive structures, and life cycles of the four phyla of fungi, as well as the mutually beneficial associations of certain fungi with other organisms. As we begin

The Tip of the Iceberg

These fungal fruiting bodies of *Armillariella* are only a hint of the presence of a vast underground network of microscopic filaments extending over many acres.

In our study, recall that the fungi and the animals are descended from a common ancestor—we are more closely related to molds and mushrooms than we are to the flowers we admired in the last chapter.

General Biology of the Fungi

The fungi are superbly adapted for absorptive nutrition: They secrete digestive enzymes that break down large food molecules in the environment, then absorb the breakdown products. The kingdom Fungi encompasses heterotrophic

organisms with absorptive nutrition. Many fungi are saprobes that absorb nutrients from dead matter, others are parasites that absorb nutrients from living hosts (Figure 30.1), and still others live in mutually beneficial symbioses with other organisms.

All fungi form spores, but only in one phylum (Chytridiomycota) do spores or gametes possess flagella. Fungi reproduce sexually in a variety of ways. Their cell walls contain at least some chitin, a polysaccharide that is also found in the skeletons of arthropods and in some protists. Most fungi have complex body forms.

These criteria enable us to distinguish between the fungi and some protists that resemble them. The slime molds consist of two protist groups whose members take up food



530 CHAPTER THIRTY

30.1 Parasitic Fungi Attack Other Living Organisms

(a) The gray masses on this ear of corn are the parasitic fungus *Ustilago maydis*, commonly called corn smut.

(b) The tropical fungus whose fruiting body is growing out of the carcass of this ant has developed from a spore ingested by the ant. The spores of this fungus must be ingested by insects before they will germinate and develop. The growing fungus absorbs organic and inorganic nutrients from the ant's body, eventually killing it, after which the fruiting body produces a new crop of spores. (c) An amoeba (below) being parasitized by a fungus (above) of the genus *Amoebophilus* ("amoeba lover").

by phagocytosis rather than by absorption, and a third protist group whose members have cells with flagella. Other funguslike protists (Oomycota) also have flagellated cells; they have cellulose rather than chitin in their cell walls.

The kingdom Fungi consists of four phyla: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (Table 30.1). We distinguish the phyla on the basis of their methods and structures for sexual reproduction and, to a lesser extent, on criteria such as the presence or absence of cross-walls separating their cell-like compartments. This morphologically based phylogeny has proved largely consistent with phylogenies based on DNA sequencing.

Some fungi, called imperfect fungi or deuteromycetes, do not form sexual structures by which they might be easily identified as members of one of the four phyla. However, techniques of molecular taxonomy, such as DNA sequencing, have allowed us to identify many imperfect fungi as asexual zygomycetes, ascomycetes, or basidiomycetes. The deuteromycetes are not considered a phylum, but rather a "holding group" for species whose status is yet to be resolved.

The fungi are an ancient kingdom. Fossil evidence suggests that they have been present since at least 600 million years ago, and perhaps much longer.

In the sections that follow, we'll consider some aspects of the general biology of the fungi, including their body struc-

Fungal fruiting body





I

Fungus

Amoeba

" m

12 *-WW".r; >.■>:.

&

ture and its intimate relationship with their environment, their nutrition, and some special aspects of their unusual sexual reproductive cycles.

Some fungi are unicellular

Unicellular forms are found in all of the fungal phyla, as well as among the deuteromycete group. Unicellular members of the Zygomycota, Ascomycota, and Basidiomycota are called yeasts. Yeasts may reproduce by budding, by fission, or by sexual means, which help us to place them in their appropriate phyla (Figure 30.2).

J (J t l Classification of Fungi

PHYLUM

COMMON NAME

FEATURES

EXAMPLES

Chytridiomycota Zygomycota

Ascomycota Basidiomycota

Chytrids Zygomycetes

Ascomycetes Basidiomycetes

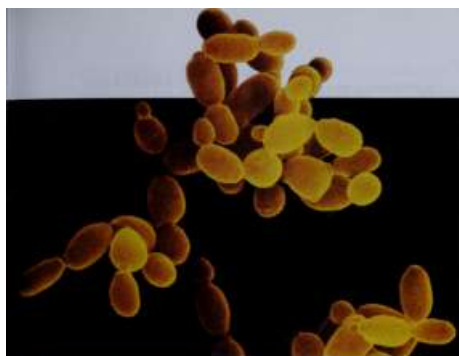
Aquatic; gametes have flagella Zygosporangium; no regularly

occurring septa; usually no

fleshy fruiting body Ascus; perforated septa Basidium; perforated septa

Allomyces Rhizopus

Neiuvspora, baker's yeast Puccinia, mushrooms



Saccharomyces sp.

30.2 Yeasts Are Microscopic, Unicellular Fungi

Unicellular members of the fungal phyla are known as yeasts. Many yeasts reproduce by budding, as those shown here are

doing.

The body of a fungus is composed of hyphae

Most fungi are not unicellular, but whether they can truly be called multicellular is questionable. The vegetative (feeding) body of a fungus is called a mycelium (plural mycelia). It is composed of rapidly growing individual tubular filaments called hyphae (singular hypha). Within most hyphae, there is no division into separate cells, and organelles (even nuclei) can move around (Figure 30.3). Thus, it may be more appropriate to call these fungi multinucleated than multicellular. Some hyphae are subdivided into cell-like compartments by incomplete cross-walls called septa (singular septum). Other hyphae are coenocytic and have no septa.

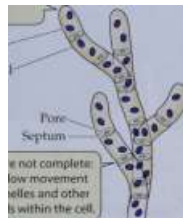
(a) Coenocytic hypha

(b) Septate hypha

Both types of hyphae are single cells with multiple nuclei.



Nuclei Cell wall



Pore Septum

Septa are not complete: Pores allow movement of organelles and other materials within the cell.

30.3 Most Hyphae Are Not Divided into Separate Cells

Even when septa are present, they do not block the movement of organelles within the hypha.

FUNGI: RECYCLERS, KILLERS, AND PLANT PARTNERS 531

Certain modified hyphae, the rhizoids, anchor Chytrid-omycota to their substrate (the dead organism or other matter upon which they feed). These rhizoids are not homologous to the rhizoids of plants. Parasitic fungi may have modified hyphae that take up nutrients from their host.

The total hyphal growth of a mycelium (not the growth of an individual hypha) may exceed 1 km per day. The hyphae may be highly dispersed or may clump together in a cottony mass. Sometimes, when sexual spores are produced, the mycelium becomes organized into elaborate fruiting bodies such as mushrooms.

The way in which a parasitic fungus attacks a plant illustrates the roles of some fungal structures (Figure 30.4). The hyphae of a fungus invade a leaf through the stomata, through wounds, or in some cases, by direct penetration of epidermal cells. Once inside the leaf, the hyphae form a mycelium. Some hyphae grow into the living plant cells, absorbing the nutrients within the cells. Fruiting bodies may form, either within the plant body or on its surface.

Fungi are in intimate contact with their environment

The tubular hyphae of a fungus give it a unique relationship with its physical environment. The fungal mycelium has an enormous surface area-to-volume ratio compared with that of most large multicellular organisms. This large ratio of surface area to volume is a marvelous adaptation for absorptive nutrition. Throughout the mycelium (except in fruiting bodies), all the hyphae are very close to their environmental food source.

Another characteristic of some fungi is their tolerance for highly hypertonic environments (those with a solute concentration higher than their own; see Chapter 5). Many fungi are more resistant than bacteria to damage in hypertonic surroundings. Jelly in the refrigerator, for example, will not become a growth medium for bacteria, because it is too hypertonic to the bacteria, but it may eventually harbor mold colonies. The refrigerator itself illustrates another trait of many fungi: tolerance of temperature extremes. Many fungi tolerate temperatures as low as 5-6°C below freezing, and some tolerate temperatures as high as 50°C or more.

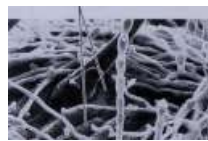
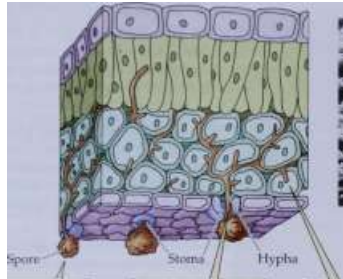
Fungi are absorptive heterotrophs

All fungi are heterotrophs that obtain food by direct absorption from their immediate environment. The majority are saprobes, obtaining their energy, carbon, and nitrogen directly from dead organic matter through the action of enzymes they secrete. However, as we've learned already, some are parasites, and still others form mutualistic associations with other organisms.

Saprobic fungi, along with bacteria, are the major decomposers of the biosphere, contributing to decay and thus to the recycling of the elements used by living things. In the forest, for example, the invisible mycelia of fungi absorb nutrients from fallen trees, thus decomposing their wood. Fungi are the principal decomposers of cellulose and lignin,

532 CHAPTER THIRTY

Grass cells



wm&fei

:^4C

Q Fungal spores germinate on the surface of the leaf

fp Elongating hyphae pass through stomata into the interior of the leaf.

§|Some hyphae penetrate cells within the leaf.

the main components of plant cell walls (most bacteria cannot break down these materials).

Because many saprobic fungi are able to grow on artificial media, we can perform experiments to determine their exact nutritional requirements. Sugars are their favored source of carbon. Most fungi obtain nitrogen from proteins or the products of protein breakdown. Many fungi can use nitrate (NO_3^-) or ammonium (NH_4^+) ions as their sole source of nitrogen. No known fungus can get its nitrogen directly from nitrogen gas, as can some bacteria and plant-bacteria associations (see Chapter 36). Nutritional studies also reveal that most fungi are unable to synthesize their own thiamin (vitamin B₂) or biotin (another B vitamin), and must absorb these vitamins from their environment. On the other hand, fungi can synthesize some vitamins that animals cannot. Like all organisms, fungi also require some mineral elements.

Nutrition in the parasitic fungi is particularly interesting to biologists. Facultative parasites can be grown by themselves on defined artificial media. Obligate parasites cannot be grown on any available medium; they can grow only on their specific living hosts, usually plants. Because their growth is limited to living hosts, they must have unusual nutritional requirements.

Some fungi have adaptations that enable them to function as active predators, trapping nearby microscopic protozoists or animals, from which they obtain nitrogen and energy. The most common strategy is to secrete sticky substances from the hyphae so that passing organisms stick tightly to them. The hyphae then quickly invade the prey, growing and branching within it, spreading through its body, absorbing nutrients, and eventually killing it.

A more dramatic adaptation for predation is the constricting ring formed by some species of *Arthrobotrys*, *Dactylaria*, and *Dactylella* (Figure 30.5). All of these fungi grow in soil. When nematodes (tiny roundworms) are present in the soil, these fungi form three-celled rings with a di-



30.4 A Fungus Attacks a Leaf

The white structures in the micrograph are hyphae of the fungus *Blumeria graminis*, which is growing on the dark surface of the leaf of a grass.

'Fungal hyphae

ameter that just fits a nematode. A nematode crawling through one of these rings stimulates it, causing the cells of the ring to swell and trap the worm. Fungal hyphae quickly invade and digest the unlucky victim.

Certain highly specific associations between fungi and other organisms have nutritional consequences for the fungal partner. Lichens are associations of a fungus with a cyanobacterium, a unicellular photosynthetic eukaryote, or both. Mycorrhizae (singular mycorrhiza) are associations between specific fungi and the roots of plants. In such associations the fungus obtains organic compounds from its photosynthetic partner, but provides it with minerals and water so that the partner's nutrition is also promoted. We will discuss lichens and mycorrhizae more thoroughly later in this chapter.

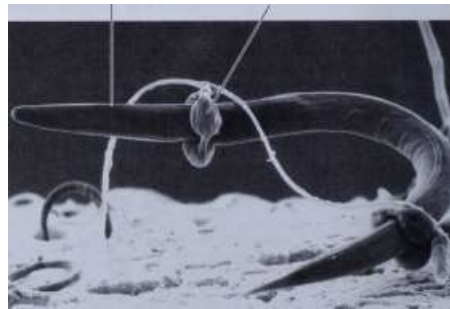
Most fungi reproduce both asexually and sexually

Both asexual and sexual reproduction are common among the fungi. Asexual reproduction takes several forms:

- The production of (usually) haploid spores within structures called sporangia.

Roundworm

Fungal loop



30.5 Some Fungi Are Predators

A nematode (roundworm) is trapped in sticky loops of the soil-dwelling fungus *Arthrobotrys anthonia*.

- The production of naked spores (not enclosed in sporangia) at the tips of hyphae; such spores are called conidia (from the Greek *kōnis*, "dust").
- Cell division by unicellular fungi—either a relatively equal division or an asymmetrical division in which a tiny bud is produced.
- Simple breakage of the mycelium.

Sexual reproduction in many fungi features an interesting twist. There is often no morphological distinction between female and male structures, or between female and male individuals. Rather, there is a genetically determined distinction between two or more mating types. Individuals of the same mating type cannot mate with one another, but they can mate with individuals of another mating type. This distinction prevents self-fertilization. Individuals of different mating types differ genetically from one another, but are often visually and behaviorally indistinguishable. Many protists also have mating type systems.

In many fungi, the zygote nuclei formed by sexual reproduction are the only diploid nuclei in the life cycle. These nuclei undergo meiosis, producing haploid nuclei that become incorporated into spores. Haploid fungal spores, whether produced sexually in this manner or asexually, germinate, and their nuclei divide mitotically to produce hyphae.

Many fungal life cycles include a dikaryon stage

The hyphae of some Zygomycota, Ascomycota, and Basidiomycota have a nuclear configuration other than the familiar haploid or diploid. In these fungi, sexual reproduction begins in an unusual way: The cytoplasm of two individuals of opposite mating types fuse (plasmogamy) long before their nuclei fuse (karyogamy), so that two genetically different haploid nuclei exist within the same hypha. This hypha is called a dikaryon (having two nuclei). Because the two nuclei differ genetically, the hypha is also called a heterokaryon (having different nuclei).

Eventually, specialized fruiting structures form, within which the pairs of dissimilar nuclei—one from each parent—fuse, giving rise to zygotes long after the original "mating." The zygote nucleus undergoes meiosis, producing four haploid nuclei. The mitotic descendants of those nuclei become the nuclei of the next generation of hyphae.

The reproduction of such fungi displays several unusual features. First, there are no gamete cells, only gamete nuclei. Second, there is never any true diploid tissue, although for a long period the genes of both parents are present in the dikaryon and can be expressed. In effect, these hyphae are neither diploid ($2n$) nor haploid (n); rather, they are dikaryotic ($n + n$). A harmful recessive mutation in one nucleus may be compensated for by a normal allele on the same chromosome in the other nucleus. Dikaryosis is perhaps the most significant of the genetic peculiarities of the fungi.

Finally, although Zygomycota, Ascomycota, and Basidiomycota grow in moist places, their gamete nuclei are not motile and are not released into the environment. Therefore, liquid water is not required for fertilization.

Some fungi are pathogens

Fungal pathogens are a major cause of death among people with compromised immune systems. Most patients with AIDS die

of fungal diseases, such as the pneumonia caused by *Pneumocystis carinii* or the incurable diarrhea caused by some other fungi. *Candida albicans* and certain other yeasts also cause severe diseases in individuals with AIDS and in individuals taking immunosuppressive drugs. Such fungal diseases are a growing international health problem. Our limited understanding of the basic biology of these fungi still hampers our ability to treat the diseases they cause.

Various fungi cause other, less threatening human diseases, such as ringworm and athlete's foot. Still others are responsible for plant diseases that affect human food supplies. These diseases include black stem rust of wheat and other diseases of wheat, corn, and oats. Fungal diseases of plants have cost billions of dollars in crop losses.

Diversity in the Kingdom Fungi

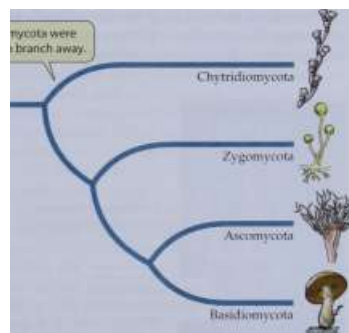
Each of the four phyla of the kingdom Fungi appears to be monophyletic (Figure 30.6). Because the imperfect fungi (deuteromycetes) are polyphyletic, we will not give them phylum status. In this section on fungal diversity, we'll consider the four phyla—Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota—and we'll discuss the status of the deuteromycetes.

Chytrids probably resemble the ancestral fungi

The earliest-diverging fungal lineage is the chytrids (phylum Chytridiomycota). These aquatic microorganisms have sometimes been classified as protists. We place chytrids among the fungi because their cell walls consist primarily of chitin and because molecular evidence indicates that they and the other fungi form a monophyletic group.

Chytridiomycota were the first to branch away.

Common ancestor



Basidiomycota

Figure 30.6 Phytogeny of the Fungi

Four phyla are recognized among the fungi. In addition, the imperfect fungi, or Deuteromycetes, functions as a "holding group" for fungal species whose status is yet to be determined.



534 CHAPTER THIRTY



Zygomycota

Ascomycota

Basidiomycota

Chytrids are either parasitic (on organisms such as algae, mosses, and nematodes) or saprobic, obtaining nutrients by breaking down dead organic matter. (Chytrids in the compound stomachs of foregut-fermenting animals may be an exception, living in a mutualistic association with their hosts.) Most chytrids live in freshwater habitats or in moist soil, but some are marine. Some chytrids are unicellular; others have mycelia made up of branching chains of cells. Chytrids reproduce both sexually and asexually, but they do not have a dikaryon stage.

Allomyces, a well-studied genus of chytrids, displays alternation of generations. A haploid zoospore (spore with flagella) comes to rest on dead plant or animal material in water and germinates to form a small haploid organism. That produces female and male gametangia (gamete cases) (Figure 30.7). The male gametangia are smaller than the female gametangia and possess a light orange pigment. Mitosis in the gametangia results in the formation of haploid gametes, each with a single nucleus.

Both female and male gametes have flagella. The motile female gamete produces a pheromone, a chemical that attracts the swimming male gamete. The gametes fuse in pairs, and then their nuclei fuse to form a diploid zygote. Mitosis and cytokinesis in the zygote gives rise to a small diploid organism, which produces numerous diploid flagellate zoospores. These diploid zoospores disperse and germinate to form more diploid organisms. Eventually, the diploid organism produces thick-walled resting sporangia that can survive unfavorable conditions such as dry weather or freezing. Nuclei in the resting sporangia

eventually undergo meiosis, giving rise to haploid zoospores that are released into the water and begin the cycle anew.

Chytrids are the only fungi that have flagella at any life cycle stage. We speculate that the protist ancestor of the fungi possessed flagella, because the phylum Chytridiomycota



The female gametangia contain female gametes.

male gametangia contain male gametes.

Allomyces sp.

30.7 Reproductive Structures of a Chytrid

The haploid gametes produced in these gametangia will fuse with another gamete to form a diploid organism.

cota was the first fungal group to diverge from the others (see Figure 30.6). The same protist ancestor gave rise to the protist group Choanoflagellida and to the animal kingdom. A key event in the evolution of the fungi after the chytrids diverged from the others was the loss of flagella.

Julm Zygomycetes reproduce sexually by fusion Vfljv of two gametangia

Most zygomycetes (phylum Zygomycota) have coenocytic hyphae (hyphae without regularly occurring septa). They produce no motile cells, and only one diploid cell—the zygote—appears in the entire life cycle. The mycelium of a zygomycete spreads over its substrate, growing forward by means of specialized hyphae. Most zygomycetes do not form a fleshy fruiting body; rather, the hyphae spread in an apparently random fashion, with occasional stalked sporangio-phores reaching up into the air (Figure 30.8).

Almost 900 species of zygomycetes have been described. A very important group of zygomycetes serves as the fungal partners in the most common type of mycorrhizal association with plant roots. A zygomycete that you may be more familiar with is Rhizopus stolonifer, the black bread mold. Rhizopus reproduces asexually by producing many stalked sporangiophores, each bearing a single sporangium containing hundreds of minute spores (Figure 30.9f). Other zygomycetes have sporangiophores with many sporangia. As in other filamentous fungi, the spore-forming structure is separated from the rest of the hypha by a wall.

Zygomycetes reproduce sexually when adjacent hyphae of two different mating types release pheromones, which cause them to grow together. These hyphae produce ga-

Chytridiomycota

Ascomycota

Basidiomycota



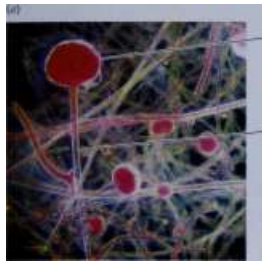
Sporangia

Sporangiophores

Phycomyces sp.

30.8 A Zygomycete

This small forest of filamentous structures is made up of sporangiophores. The stalks end in tiny, rounded sporangia.



Sporangium

Rhizopus sp.

30.9 Sexual Reproduction in a Zygomycete

(a) Sporangiphores have sprouted from the zygospores of a bread mold. (b) Sexual reproduction in zygomycetes occurs when pheromones released by two different mating types cause them to fuse and form zygosporangia.

(b)

Sporangiophore

| Hyphae of differing mating types produce branches that grow toward each other.

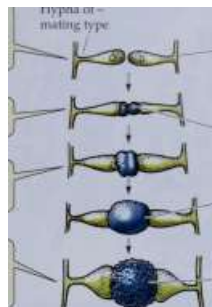
t

The tips develop into gametangia.

| The gametangia—and then the gametes within them—fuse.

o The resulting zygote develops into a zygosporangium that contains zygospores.

Hypha of - mating type



Hypha of + mating type

Gametangia (n)

Zygosporangium (n + n)

Zygospores {In}

within

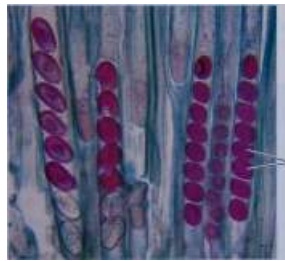
zygosporangium

metangia that fuse to form zygosporangia containing zygospores (Figure 30. 9b). The zygosporangia develop thick, multilayered walls that protect the zygospores. The highly resistant zygospores may remain dormant for months before their nuclei undergo meiosis and a sporangium sprouts. The sporangium contains the products of meiosis: haploid nuclei that are incorporated into spores. These spores disperse and germinate to form a new generation of haploid hyphae.

The sexual reproductive structure of ascomycetes is an ascus

The ascomycetes (phylum Ascomycota) are a large and diverse group of fungi distinguished by the production of sacs called asci (singular ascus) (Figure 30.10). The ascus is the characteristic sexual reproductive structure of the ascomycetes. Ascomycete hyphae are segmented by more or

Ascus



Ascospores

30.10 Asci and Ascospores

The ascomycetes are characterized by the production of ascospores within sacs called asci. Ascospores are the products of meiosis followed by a single mitotic division. Ascospores and asci do not mature all at once, and they may abort, so not every ascus in this micrograph contains eight mature ascospores.

less regularly spaced septa. A pore in each septum permits extensive movement of cytoplasm and organelles (including the nuclei) from one segment to the next.

The approximately 30,000 known species of ascomycetes can be divided into two broad groups, depending on whether the asci are contained within a specialized fruiting structure. Species that have this fruiting structure, the ascocarp, are collectively called euascomycetes ("true ascomycetes"); those without ascocarps are called hemi-ascomycetes ("half ascomycetes").

Chytridiomycota

Zygomycota

Basidiomycota

hemiascomycetes. Most hemiascomycetes are microscopic, and many species are unicellular. Perhaps the best known are the ascomycete yeasts, especially baker's or brewer's yeast (*Saccharomyces cerevisiae*; see Figure 30.2). These yeasts are among the most important domesticated fungi. *S. cerevisiae* metabolizes glucose obtained from its environment to ethanol and carbon dioxide. It forms carbon dioxide bubbles in bread dough and gives baked bread its light texture. Although baked away in bread making, the ethanol and carbon dioxide are both retained in beer. Other yeasts live on fruits such as figs and grapes and play an important role in the making of wine.

Hemiascomycete yeasts reproduce asexually either by fission or by budding (the outgrowth of a new cell from the surface of an old one; see Figure 30.2). Sexual reproduction takes place when two adjacent haploid cells of opposite mating types fuse. (We discussed the genetics of yeast mating types in Chapter 14.) In some species, the resulting zygote buds to form a diploid cell population; in others, the zygote nucleus undergoes meiosis immediately. When these diploid nuclei undergo meiosis, the entire cell becomes an ascus. Depending on whether the products of meiosis then undergo mitosis, a yeast ascus usually has either eight or four ascospores (see Figure

536 CHAPTER THIRTY



(a) Man

lent a

30.11 Two Ascomycetes

(a) Morels, which have spongelike caps and a subtle flavor, are considered a delicacy by humans. The brilliant red cups in (b) are cup fungi, as are the three yellow morels in (a).

30.10). The ascospores germinate to become haploid cells. Hemiascomycetes have no dikaryon stage.

Yeasts, especially *Saccharomyces cerevisiae*, are frequently used in molecular biological research. Just as *E. coli* is the best-studied prokaryote, *S. cerevisiae* is the most completely studied eukaryote.

euscomycetes. The euscomycetes include the filamentous fungi known as molds. Among them are several common molds, including *Neurospora*, the pink molds, one of which Beadle and Tatum used in their pioneering work on biochemical genetics (see Figure 12.1). Many euscomycetes are parasites on higher plants. Chestnut blight and Dutch elm disease are caused by euscomycetes. The powdery mildews are euscomycetes that infect cereal grains, lilacs, and roses, among many other plants. They can be a serious problem to grape growers, and a great deal of research has focused on ways to control these agricultural pests.

The euscomycetes also include the cup fungi (Figure 30.11f and b). In most of these organisms the fruiting structures are cup-shaped and can be as large as several centimeters across. The inner surfaces of the cups are covered with a mixture of both sterile filaments and asci, and they produce huge numbers of spores. Although these fleshy structures appear to be composed of distinct tissue layers, microscopic examination shows that their basic organization is still filamentous—a tightly woven mycelium.

Two particularly delicious cup fungus fruiting structures are morels (Figure 30.11f) and truffles. Truffles grow underground, in a mutualistic association with the roots of some species of oaks. Europeans traditionally used pigs to find truffles because some truffles secrete a substance that has



(b) *Sarcoscypha coccinea*

an odor similar to a pig's sex attractant. Unfortunately, pigs also eat truffles, so dogs are now the usual truffle hunters.

Penicillium is a genus of green molds, of which some species produce the antibiotic penicillin, presumably for defense against competing bacteria. Two species, *P. camemberti* and *P. roquefortii*, are the organisms responsible for the characteristic flavors of Camembert and Roquefort cheeses, respectively.

Brown molds of the genus *Aspergillus* are important in some human diets. *A. tamarii* acts on soybeans in the production of soy sauce, and *A. oryzae* is used in brewing the Japanese alcoholic beverage sake. Some species of *Aspergillus* that grow on nuts such as peanuts and pecans produce extremely carcinogenic (cancer-inducing) compounds called aflatoxins.

The euscomycetes reproduce asexually by means of mating structures called conidia that form at the tips of specialized hyphae (Figure 30.12). Small chains of conidia are

30.72 Conidia

Chains of conidia are developing on stalks called hyphae arising from this powdery mildew growing on a leaf.

Erysiphe sp.



Conidia

Leaf

Hyphae

I Mating structures on the hyphae of two different mating types fuse.

HAPLOID(n)

Mating type A (•)

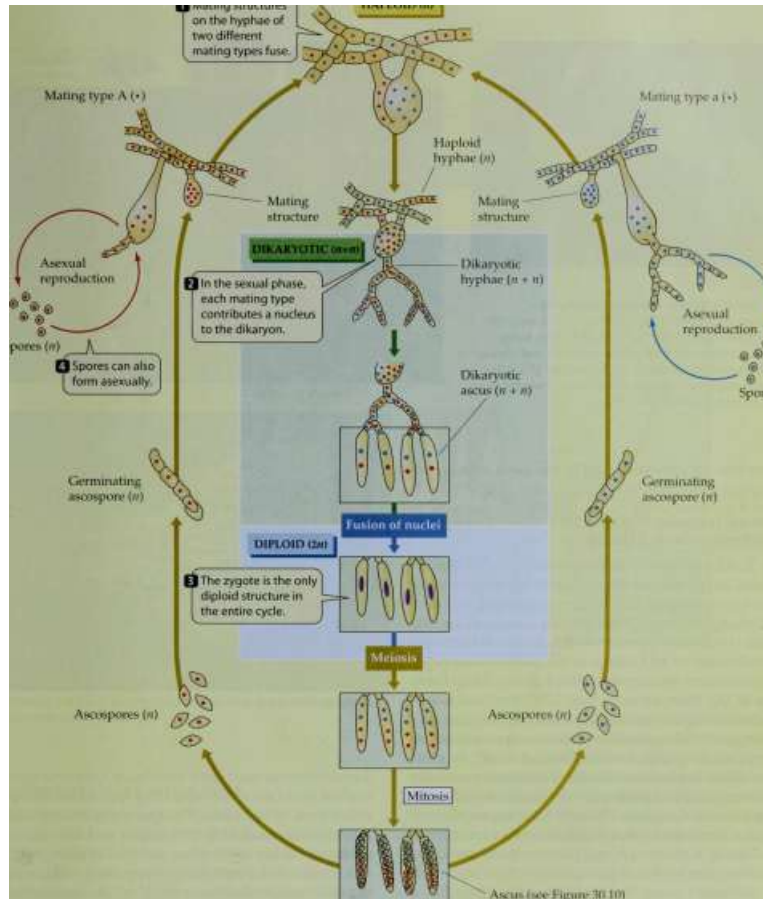
Mating type a (•)

Spores (n)

Asexual reproduction

• • •

® ® © Spores («)



Ascospores («) /T)/^.

Ascospores (») /*7 A^

Ascus (see Figure 30.10)

produced by the millions and can survive for weeks in nature. The conidia are what give molds their characteristic colors.

The sexual reproductive cycle of euascomycetes includes the formation of a dikaryon (Figure 30.13). Most euascomycetes form mating structures, some "female" and some "male."

Nuclei from a male structure on one hypha enter a female mating structure on a hypha of a compatible mating type. Dikaryotic ascogenous (ascus-forming) hyphae de-



30. 7 3 The Life Cycle of a Euascomycete

Neurospora crassa, the species represented by this life cycle, is a bread mold that is often used in genetics experiments.

velop from the now dikaryotic female mating structure. The introduced nuclei divide simultaneously with the host nuclei. Eventually asci form at the tips of the ascogenous hyphae. Only with the formation of asci do the nuclei finally fuse. Both nuclear fusion and the subsequent meiosis of the resulting diploid nucleus take place within individual asci. The meiotic products are incorporated into as-



(a) *Lycoperdon perlatum*

30.14 Basidiomycete Fruiting Structures

The fruiting structures of the basidiomycetes are probably the most familiar structures produced by fungi, (a) When raindrops hit them, these puffballs will release clouds of spores for dispersal, (b) A member of a highly poisonous mushroom genus, *Amanita*. (c) This edible bracket fungus is parasitizing a tree.

cospores that are ultimately shed by the ascus to begin the new haploid generation.

The sexual reproductive structure of basidiomycetes is a basidium

About 25,000 species of basidiomycetes (phylum Basidiomycota) have been described. Basidiomycetes produce some of the most spectacular fruiting structures found anywhere among the fungi. These fruiting structures include puffballs (which may be more than half a meter in diameter), mushrooms of all kinds, and the giant bracket fungi often encountered on trees and fallen logs in a damp forest (Figure 30.14). There are more than 3,250 species of mushrooms, including the familiar *Agaricus bisporus* you may enjoy on your pizza, as well as poisonous species, such as members of the genus *Amanita*. Bracket fungi do great damage to cut lumber and stands of timber. Some of the most damaging plant pathogens are basidiomycetes, including the smut fungi (see Figure 30.1a) that parasitize cereal grains. In contrast, other basidiomycetes contribute to the well-being of plants as fungal partners in mycorrhizae.

Basidiomycete hyphae characteristically have septa with small, distinctive pores. The basidium (plural basidia), a swollen cell at the tip of a hypha, is the characteristic sexual reproductive structure of the basidiomycetes. It is the site of nuclear fusion and meiosis. Thus, the basidium plays the same role in the basidiomycetes as the ascus does in the ascomycetes and the zygosporangium does in zygomycetes.

The life cycle of the basidiomycetes is shown in Figure 30.15. After nuclei fuse in the basidium, the resulting



Chytridiomycota

Zygomycota

Ascomycota

•+i-n.ii,i,mui

(b) *Amanita muscaria*



(c) *Laetiporus sulphureus*

diploid nucleus undergoes meiosis, and the four resulting haploid nuclei are incorporated into haploid basidiospores, which

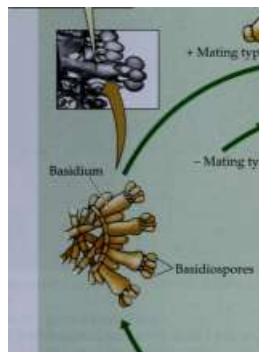
form on tiny stalks. These basidiospores typically are forcibly discharged from their basidia and then germinate, giving rise to haploid hyphae. As these hyphae grow, haploid hyphae of different mating types meet and fuse, forming dikaryotic hyphae, each cell of which contains two nuclei, one from each parent hypha. The dikaryotic mycelium grows and eventually produces fruiting structures. The dikaryotic phase may persist for years—some basidiomycetes live for decades or even centuries.

The elaborate fruiting structure of some fleshy basidiomycetes, such as the gill mushroom in Figure 30.15, is topped by a cap, or pileus, which has structures called gills on its underside. Enormous numbers of basidia develop on the surfaces of the gills. The basidia discharge their spores into the air spaces between adjacent gills, and the spores sift down into air currents for dispersal and germination as new haploid mycelia.

FUNGI: RECYCLERS, KILLERS, AND PLANT PARTNERS 539

The basidium is the characteristic sexual reproductive structure of the basidiomycetes.

Basidiospores give rise to haploid hyphae.



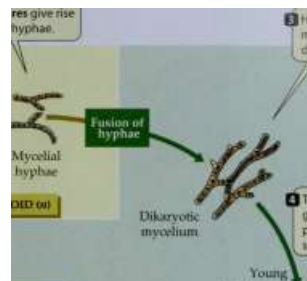
+ Mating type Basidiospores

■ * K ^ ^

^ - ^ 4 f * Mycelial p / ^ / ^ hyphae

HAPLOID (w) |

Mating type



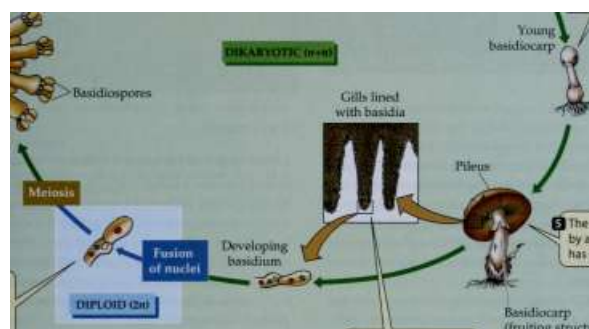
Haploid hyphae of different mating type fuse, forming dikaryotic hyphae.

Dikaryotic mycelium

The dikaryotic mycelium grows and eventually produces a fruiting structure, the basidiocarp.

Basidiospores

Nuclear fusion and meiosis take place in the developing basidium.



Meiosis

The basidiocarp is topped by a cap, or pileus, which has gills on its underside.

T

30.15 The Basidiomycete Life Cycle

Basidiospores form on tiny stalks and are then forcibly dispersed to germinate into haploid hyphae, from which the familiar fruiting structure eventually grows.

Basidia develop on the surfaces of the gills.

Basidiocarp (fruiting structure)

Imperfect fungi lack a sexual stage

Mechanisms of sexual reproduction readily distinguish members of the four phyla of fungi from one another. But many fungi, including both saprobes and parasites, lack sexual stages entirely; presumably these stages have been lost during evolution or have not yet been found. Classifying these fungi as belonging to any of the four major phyla was at one time difficult, but biologists now can classify most such fungi on the basis of DNA sequences.

Fungi that have not yet been placed in any of the existing phyla are grouped together as the imperfect fungi, or deuteromycetes. Thus, the deuteromycete group is a holding area for species whose status is yet to be resolved. At present, about 25,000 species are classified as imperfect fungi. Some taxonomists, preferring to emphasize convenience of identification over strict phylogenetic considerations, treat the imperfect fungi as a paraphyletic phylum, Deuteromycota.

If sexual structures are found on a fungus classified as a deuteromycete, the fungus is reassigned to the appropriate phylum. That happened, for example, with a fungus that produces plant growth hormones called gibberellins. Originally classified as the deuteromycete *Fusarium moniliforme*, this fungus was later found to produce asci, whereupon it was renamed *Gibberella fujikuroi* and transferred to the phylum Ascomycota.

Fungal Associations

Earlier in this chapter we mentioned mycorrhizae and lichens, in which fungi live in intimate association with other organisms. Now that we have learned a bit about fungal diversity, let's consider mycorrhizae and lichens in greater detail.

Mycorrhizae are essential to many plants

Almost all tracheophytes enjoy a mutually beneficial symbiotic association with fungi. Unassisted, the root hairs of such plants do not absorb enough water or minerals to sustain maximum growth. However, the roots usually become infected with fungi, forming an association called a mycorrhiza.

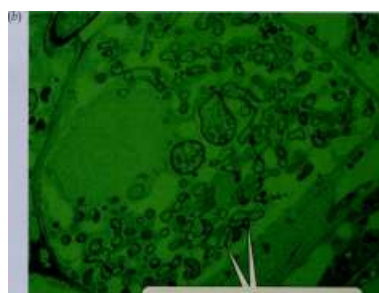
540 CHAPTER THIRTY

30.76 Mycorrhizal Associations

(a) Ectomycorrhizal fungi wrap themselves around the plant root, increasing the area available for absorption of water and nutrients, (b) Endomycorrhizae infect the root internally.



Hyphae of the fungus *Pisolithus tinctorius* cover a eucalyptus root.



The shapes filling much of this soybean root cell are sections through the hyphae of the endomycorrhizal fungus *Glomus*

caledonius.

In ectomycorrhizae, the fungus wraps around the root, and its mass is often as great as that of the root itself (Figure 30.16rt). The hyphae of the fungi attached to the root increase the surface area for the absorption of water and minerals, and the mass of the mycorrhiza, like a sponge, holds water efficiently in the neighborhood of the root. Infected roots characteristically branch extensively and become swollen and club-shaped. In endomycorrhizae, the infection is internal to the root, with no hyphae visible on the root surface (Figure 30.16b).

The symbiotic fungus-plant association of a mycorrhiza is important to both partners. The fungus obtains important organic compounds, such as sugars and amino acids, from the plant. In return, the fungus greatly increases the absorption of water and minerals (especially phosphorus) by the plant. The fungus may also provide certain growth hormones, and may protect the plant against attack by microorganisms. Plants that have active mycorrhizae typically are a deeper green and may resist drought and temperature extremes better than plants of the same species that have little mycorrhizal development. Attempts to introduce some plant species to new areas have failed until a bit of soil from the native area (presumably containing the fungus necessary to establish mycorrhizae) was provided.

The partnership between plant and fungus results in a plant better adapted for life on land. It has been suggested that the evolution of this symbiotic association was the single most important step leading to the colonization of the terrestrial environment by living things. Fossils of mycorrhizal structures more than 300 million years old have been found, and some rocks dating back 460 million years contain structures that appear to be fossilized fungal spores.

Some liverworts, which are among the most ancient terrestrial plants (see Chapter 28), form mycorrhizae. Certain plants that live in nitrogen-poor habitats, such as cranberry bushes and orchids, invariably have mycorrhizae. Orchid seeds will not germinate in nature unless they are already infected by the fungus that will form their mycorrhizae. Plants that lack chlorophyll always have mycorrhizae,

which they often share with the roots of green, photosynthetic plants.

Lichens grow where no eukaryote has succeeded

A lichen is not a single organism, but rather a meshwork of two radically different organisms: a fungus and a photo-synthetic microorganism. Together the organisms constituting a lichen can survive some of the harshest environments on Earth. The flora of Antarctica, for example, features more than 100 times as many species of lichens as of plants.

In spite of this hardiness, lichens are very sensitive to air pollution because they are unable to excrete toxic substances that they absorb. Hence they are not common in industrialized cities. Because of their sensitivity, lichens are good biological indicators of air pollution.

The fungal components of most lichens are ascomycetes, but some are basidiomycetes or imperfect fungi (only one zygomycete serving as the fungal component of a lichen has been reported). The photosynthetic component may be either a cyanobacterium or a unicellular green alga. Relatively little experimental work has focused on lichens, perhaps because they grow so slowly—typically less than 1 centimeter per year.

There are about 13,500 "species" of lichens; their fungal components may constitute as many as 20 percent of all fungal species. Lichens are found in all sorts of exposed habitats: on tree bark, open soil, or bare rock. Reindeer "moss" (actually not a moss at all, but the lichen *Cladonia subtenuis*) covers vast areas in arctic, subarctic, and boreal regions, where it is an important part of the diets of reindeer and other large mammals. Lichens come in various forms and colors. Crustose (crustlike) lichens look like colored powder dusted over their substrate (Figure 30.17a); foliose (leafy) and fruticose (shrubby) lichens may have complex forms (Figure 30.17b).

The most widely held interpretation of the lichen relationship is that it is a type of mutually beneficial symbiosis. The hyphae of the fungal mycelium are tightly pressed against the photosynthetic cells of the alga or cyanobacterium and sometimes even invade them. The bacterial or



»!■».>>'■Wit1T*MJMSMMI■?■?>»—?.^•giv

(«)

30.7 7 Lichen Body Forms

Lichens fall into three principal classes based on their body form. (a) Foliose and crustose lichens grow on otherwise bare rock, (b) A miniature jungle of fruticose lichens.

algal cells not only survive these indignities, but continue their growth and photosynthesis. In fact, algal cells in a lichen "leak" photosynthetic products at a greater rate than do similar cells growing on their own. On the other hand, photosynthetic cells from lichens grow more rapidly on their own than when combined with a fungus. On this basis, we could consider lichen fungi as parasitic on their photosynthetic partners.

Lichens can reproduce simply by fragmentation of the vegetative body, which is called the thallus, or by means of

specialized structures called soredia (singular soredium). Soredia consist of one or a few photosynthetic cells surrounded by fungal hyphae (Figure 30.1877). The soredia become detached, are dispersed by air currents, and upon arriving at a favorable location, develop into a new lichen. Alternatively, if the fungal partner is an ascomycete or a basidiomycete, it may go through its sexual cycle, producing either ascospores or basidiospores. When these spores are discharged, however, they disperse alone, unaccompanied by the photosynthetic partner, and thus may not be capable of reestablishing the lichen association. Nevertheless, many lichens produce characteristic fruiting structures containing asci or basidia.

Visible in a cross section of a typical foliose lichen are a tight upper region of fungal hyphae, a layer of cyanobacte-

30.78 Lichen Anatomy

(a) Soredia of a fruticose lichen, (b) Cross section showing the layers of a foliose lichen.

(a)

ECS

(b)



Hyphae

Lichens are arranged in distinct layers.

Soredium

Each soredium consists of one or a few photosynthetic cells surrounded by fungal hyphae.

Upper layer of fungal hyphae

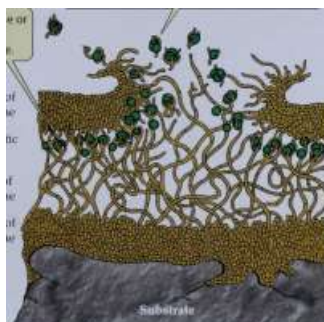
Photosynthetic cell layer

Loose layer of fungal hyphae

Lower layer of fungal hyphae

Soredia detach readily from the parent lichen and travel in air currents, founding new lichens when they settle in a suitable environment.

L&3&SI



ria or algae, a looser hyphal layer, and finally hyphal rhizoids that attach the whole structure to its substrate (Figure 30.15). The meshwork of fungal hyphae takes up some nutrients needed by the photosynthetic cells and provides a suitably moist environment for them by holding water tenaciously. The fungi derive fixed carbon from the photosynthesis of the algal or cyanobacterial cells.

Lichens are often the first colonists on new areas of bare rock. They satisfy most of their needs from the air and from rainwater, augmented by minerals absorbed from dust. A lichen begins to grow shortly after a rain, as it begins to dry. As it grows the lichen acidifies its environment slightly, and this acid contributes to the slow breakdown of rocks, an early step in soil formation. After further drying, the lichen's photosynthesis ceases. The water content of the lichen may drop to less than 10 percent of its dry weight, at which point it becomes highly insensitive to extremes of temperature.

Whether living on their own or in symbiotic associations, fungi have spread successfully over much of Earth since their origin from a protist ancestor. That ancestor also gave rise to the choanoflagellates and the animal kingdom, the group we'll consider in the next three chapters.

Chapter Summary

- Fungi are the principal degraders of dead organic matter in the biosphere. Fungi are nutritional partners of almost all eukaryotic plants. Some fungi are serious pathogens of plants and animals, including humans.

General Biology of the Fungi

- Fungi are heterotrophic eukaryotes with absorptive nutrition. They may be saprobes, parasites, or mutualists. Review Figure 30.4
- The yeasts are unicellular.
- The bodies of other fungi are composed of chitinous-walled, multinucleate hyphae, often massed to form a mycelium. The filamentous hyphae give fungi a large surface area-to-volume ratio, enhancing their ability to absorb nutrients. The hyphae usually have incomplete partitions (septa) that do not divide them into separate cells. Review Figure 30.5
- Fungi reproduce asexually by means of spores formed within sporangia, by conidia formed at the tips of hyphae, by budding, or by fragmentation.
- Fungi reproduce sexually when hyphae or motile cells of different mating types meet and fuse.
- In addition to the haploid and diploid states, many fungi demonstrate a third nuclear condition: the dikaryotic, or

Stable Review Figure 30.13

Diversity in the Kingdom Fungi

- The kingdom Fungi consists of four phyla: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. These phyla differ in their reproductive structures, mechanisms of reproduction, and less importantly, the presence and form of their hyphae. Review Figure 30.6, Table 30.1
- Chytrids, with their flagellated zoospores and gametes, probably resemble the ancestral fungi.
- Zygomycetes reproduce sexually by fusion of gametangia. Review Figure 30.9
- The sexual reproductive structure of ascomycetes is an ascus containing ascospores. The ascomycetes are divided into two groups, euascomycetes and hemiascomycetes, on the basis of whether they have an ascocarp, or fruiting structure. Review Figure 30.13
- The sexual reproductive structure of basidiomycetes is a basidium, a swollen cell bearing basidiospores. Review Figure 30.15
- Imperfect fungi (deuteromycetes) lack sexual structures, but DNA sequencing can sometimes identify the phylum to which they belong.

Fungal Associations

- Mycorrhizae, associations of fungi with plant roots, enhance the ability of the roots to absorb water and nutrients.
- Lichens, mutualistic combinations of a fungus with a cyanobacterium or a green alga, are found in some of the most inhospitable environments on the planet. Review Figure 30.18

For Discussion

1. You are shown an object that looks superficially like a pale green mushroom. Describe at least three criteria (including anatomical and chemical traits) that would enable you to tell whether the object is a piece of a plant or a piece of a fungus.
2. Differentiate among the members of the following pairs of related terms:
 - a. hypha/mycelium
 - b. euascomycete/hemiascomycete
 - c. ascus/basidium
 - d. ectomycorrhiza/endomycorrhiza
3. For each type of organism listed below, give a single characteristic that may be used to differentiate it from the other, related organism(s) in parentheses.
 - a. Zygomycota (Ascomycota)
 - b. Basidiomycota (deuteromycetes)
 - c. Ascomycota (Basidiomycota)
 - d. baker's yeast {*Neurospora crassa*}
4. Many fungi are dikaryotic during part of their life cycle. Why are dikaryons described as $n + n$ instead of $2n$?
5. If all the fungi on Earth were suddenly to die, how would the surviving organisms be affected? Be thorough and specific in your answer.
6. How might the first mycorrhizae have arisen?
7. What might account for the ability of lichens to withstand the intensely cold environment of Antarctica? Be specific in your answer.

31

Animal Origins and Lophotrochozoans



In 1822, NEARLY 40 YEARS BEFORE DARWIN wrote *The Origin of Species*, French naturalist E. Geoffroy Saint-Hilaire was examining a lobster. He noticed that when he viewed the lobster with its ventral surface up, its central nervous system was located above its digestive tract, which in turn was located above its heart—the same relative positions these systems have in mammals viewed dorsally. His observation led Saint-Hilaire to conclude that the differences between arthropods and vertebrates could be explained if the embryos of one of those groups had been inverted during development.

Saint-Hilaire's suggestion was regarded as totally preposterous at the time and was largely dismissed until recently. However, the discovery of two genes that influence a system of extracellular signals involved in development has lent new support to Saint-Hilaire's seemingly outrageous hypothesis.

A vertebrate gene called *chordin* helps establish cells on one side of the embryo as dorsal, the other as ventral. A probably homologous gene in fruit flies,* called *sog*, acts in a similar manner, but has the opposite effect. Fly cells where *sog* is active become ventral, whereas vertebrate cells expressing *chordin* become dorsal (see Figure 16.19). However, when *sog* mRNA is injected into the frog *Xenopus*, a vertebrate, it causes dorsal development. *Chordin* mRNA injected into flies promotes ventral development. In both cases, injection of the mRNA promotes the development of the portion of the embryo that contains the central nervous system!

Chordin and *sog* are among the many genes that appear to regulate similar functions in very different organisms. There

"Insects (such as fruit flies) are arthropods and belong to the same evolutionary lineage as crustaceans (such as the lobster), as we will discuss in Chapter 32.

Genes that Control Development

The human and the lobster carry similar genes that control the development of the body axis. A lobster's nervous system runs up its ventral (belly) surface, while its circulatory system is dorsal (down its back). In vertebrates such as humans, similar genes position these two systems inversely to those of the lobster.

are several almost universal animal genes that help transform a single-celled egg into a multicellular adult. Such genes are providing evolutionary biologists with information that can help them understand relationships among animal lineages that separated from one another in ancient times. As we saw in Chapter 23 new knowledge about gene functions and gene sequences provides some of the most powerful data being used in modern phylogenetic investigations to infer evolutionary relationships among organisms.

In this chapter we will first discuss how biologists infer evolutionary relationships among animals and review the defining characteristics of the animal way of life. Then we will describe several lineages of simple animals. Finally, we will describe the lophotrochozoans, one of the three great evolutionary lineages of animals. The next two chapters will discuss the other two great animal lineages: the ecdysozoans and the deuterostomes.

Descendants of a Common Ancestor

Biologists have long debated whether animals arose once or several times from protist ancestors, but enough molecular and morphological evidence has now been assembled to indicate that, with the possible exception of sponges (Porifera), the kingdom Animalia is a monophyletic group—that is, all animals are descendants of a single ancestral lineage.



544 CHAPTER THIRTY-ONE

This conclusion is supported by the fact that all animals share a set of derived traits:

- ▶ Similarities in their 5S and 18S ribosomal RNAs
- ▶ Special types of cell-cell junctions: tight junctions, desmosomes, and gap junctions (see Figure 5.6)
- ▶ A common set of extracellular matrix molecules, including collagen (see Figure 4.28)

Animals evolved from ancestral colonial flagellated protists as a result of division of labor among their aggregated cells. Within these ancestral colonies of cells—perhaps analogous to those still existing in the chlorophyte *Volvox* or some colonial choanoflagellates (see Figures 27.25a and 27.28)—some cells became specialized for movement, others for nutrition, and still others differentiated into gametes. Once the division of labor had begun, these units continued to differentiate while improving their coordination with other working groups of cells. Such coordinated groups of cells evolved into the larger and more complex organisms that we now call animals.

The Animal Way of Life

What traits characterize the organisms we call animals? Animals are multicellular organisms that must take in preformed organic molecules because they cannot synthesize them from inorganic chemicals. They acquire these organic molecules by ingesting other organisms, either living or dead, and digesting them inside their bodies. To acquire these organic molecules, animals must expend energy to move themselves through the environment to find food, to position themselves where food will pass by them, or to move the environment and the food it contains to them.

The foods animals eat include most other members of the animal kingdom, as well as members of all other evolutionary lineages. Much of the diversity of animal sizes and shapes evolved as animals acquired the ability to capture and eat many different kinds of foods, and to avoid becoming food for other animals.

(a) Acoelomate

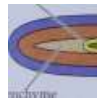


(b) Pseudocoelomate

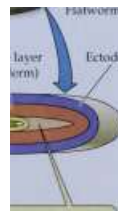
Flatworm

Gut Muscle layer

(endoderm) (mesoderm)



Mesenchyme



Ectoderm Gut (endoderm)

Pseudocoel

Muscle (mesoderm)

Internal organs

Ectoderm

The need to move in search for food has favored sensory structures that provide animals with detailed information about their environment, and nervous systems able to receive and coordinate this information. Consequently, most animals are behaviorally much more complex than plants. Because animals ingest chemically complex foods, they expend considerable energy to maintain relatively constant internal conditions while taking in foods that vary chemically.

A real appreciation of animal structure and functioning is best achieved through firsthand experience in the field and laboratory. The accounts in this chapter and the following two serve as an orientation to the major groups of animals, their similarities and differences, and the evolutionary pathways that resulted in the current richness of animal evolutionary lineages and species. But how do biologists infer evolutionary relationships among animals?

Clues to Evolutionary Relationships among Animals

Biologists use a variety of traits to infer animal phylogenies. As we discussed in Chapters 23 and 24, clues to these relationships are found in the fossil record, in patterns of embryonic development, in the comparative morphology and physiology of living and fossil animals, and in the structure of their molecules.

Patterns of early development evolved very slowly in some animal lineages. For this reason, biologists have traditionally based their classifications of the major lineages of animals on developmental patterns. More recently, comparative molecular data from small subunit rRNA and mitochondrial genes also have been used. These two types of evidence suggest similar animal phylogenies.

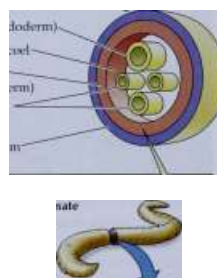
31.1 Animal Body Cavities

The three major types of animal body cavities, (a) Acoelomates do not have enclosed body cavities, (b) Pseudocoelomates have only one layer of muscle, lying outside the body cavity, (c) Coelomates have a peritoneum surrounding the internal organs; the body cavities of some, such as this earthworm, are segmented.



(c) Coelomate

Roundworm



Earthworm

Gut (endoderm)

Internal organ

Peritoneum (mesoderm)

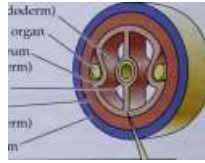
Coelom

Muscle (mesoderm)

Ectoderm

Acoelomates do not have enclosed body cavities.

Pseudocoelomates have a cavity lined with muscle (mesoderm) on the outer side, but no mesoderm surrounds the internal organs.



Coelomates possess body cavities with mesoderm on both the outer side and surrounding internal organs.

Using this wide variety of comparative data, zoologists have concluded that the sponges, cnidarians, and ctenophores separated from the remaining animal lineages early in evolutionary history. They have divided the remaining animals into two major lineages: the protostomes and the deuterostomes.

In the common ancestor of the protostomes and the deuterostomes, the pattern of early cell division in the fertilized egg—called cleavage—was radial. During radial cleavage, cells divide along a plane either parallel to or at right angles to the long axis of the fertilized egg. This pattern persisted during the evolution of deuterostomes and in many protostome lineages, but spiral cleavage evolved in one major protostome lineage. In spiral cleavage, the plane of cell division is oblique to the long axis of the egg, causing the cells to be arranged in a spiral pattern.

Other developmental patterns typically differ between protostomes and deuterostomes. Cleavage of the fertilized egg in protostomes is determinate; that is, if the egg is allowed to divide a few times and the cells are then separated, each cell develops into only a partial embryo. In contrast, cleavage in deuterostomes typically is indeterminate; cells separated after several cell divisions can still develop into complete embryos. We see this phenomenon in humans in identical twins. Among deuterostomes, the mouth of the embryo originates some distance away from the embryonic structure called the blastopore, which becomes the anus. Among protostomes, the mouth arises from or near the blastopore.

During development from a single-celled zygote to a multicellular adult, animals form layers of cells. The embryos of diploblastic animals have only two cell layers: an outer ectoderm and an inner endoderm. The embryos of triploblastic animals have a third layer, the mesoderm, which lies between the ectoderm and the endoderm.

Fluid-filled spaces, called body cavities, lie between the cell layers of the bodies of many kinds of animals. The type of body cavity an animal has strongly influences how it can move.

Common ancestor of all animals

Animals that lack an enclosed body cavity are called acoelomates. In these animals, the space between the gut and the body wall is filled with masses of cells called mesenchyme (Figure 31.1a). Another group of animals, the pseudocoelomates, have a body cavity called the pseudocoel. The pseudocoel is a liquid-filled space in which many of the body organs

37.2 A Probable Phylogeny of Animals

The evolutionary tree that we will use in this chapter and the following two postulates that animals are monophyletic. The traits highlighted by red circles on the tree will be explained as we discuss the different phyla.

Indeterminate cleavage, blind gut, two cell layers

are suspended, but control over body shape is crude because a pseudocoel has muscles only on the outside (Figure 31.1b). ► Coelomate animals have a coelom, a body cavity that develops within the embryonic mesoderm. It is lined with a special structure called the peritoneum, and has muscles both inside and outside. The internal organs of coelomates are slung in pouches of peritoneum rather than being suspended within the body cavity (Figure 31.1c).

An animal with a coelom has better control over the movement of the fluids it contains, but control is limited if the animal has only a single, large body cavity. Control is improved if the coelom is separated into compartments or segments so that circular and longitudinal muscles in each individual segment can change its shape independently of the other segments. Segmentation of the coelom evolved several different times among both protostomes and deuterostomes.

The phylogeny of animals we adopt in this book is based on analyses of many developmental, structural (from both living and fossil animals), and molecular traits. Figure 31.2 shows the postulated order of splitting of the major lineages in animal evolution. New information continues to modify and refine our understanding of the details of phylogenetic relationships among animals. Nonetheless, the division of the animals into the lineages shown here is supported by many types of data.

Body Plans Are Basic Structural Designs

The entire structure of an animal, its organ systems, and the integrated functioning of its parts are known as its body plan. Animals in many (but not all) lineages have evolved greater body complexity over time.

A fundamental aspect of an animal's body plan is its overall shape, described as its symmetry. A symmetrical animal can be divided along at least one plane into similar halves. Animals that have no plane of symmetry are said to

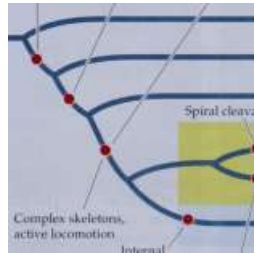
Complete gut,

determinate

cleavage

Radial cleavage, bilateral symmetry, three body layers, Hox cluster expansion

Spiral cleavage



Porifera (sponges)

Cnidaria

Ctenophora Lophotrochozoans

PROTOSTOMES

Ecdysozoans

Complex skeletons, active locomotion

DEUTEROSTOMES

Internal' skeletons

Molting of external skeletons



546 CHAPTER THIRTY-ONE

The sea star, an echinoderm, has biradial symmetry.

Main axis



37.3 Body Symmetry

Most animals are either biradially or bilaterally symmetrical. Biradially symmetrical animals appear similar to radially symmetrical ones.

Two planes, at right angles to each other, divide the animal into similar halves.

The fish, a vertebrate, has bilateral symmetry.

Dorsal
terior



Only one plane divides the animal into similar, mirror-image halves.

Vent

be asymmetrical. Many sponges are asymmetrical, but most animals have some kind of symmetry.

The simplest form of symmetry is spherical symmetry, in which body parts radiate out from a central point. An infinite number of planes passing through the central point can divide a spherically symmetrical organism into similar halves. Spherical symmetry is widespread among protists, but most animals possess other forms of symmetry.

An organism with radial symmetry has one main axis around which its body parts are arranged. A perfectly radially symmetrical animal can be divided into similar halves by any plane that contains the main axis. Some simple sponges and a few other animals, such as some sea anemones, have true radial symmetry.

Most radially symmetrical animals are modified such that only two planes, at right angles to each other, can divide them into similar halves. These animals are said to have biradial symmetry (Figure 31.3a). Three animal phyla—Cnidaria, Ctenophora, and Echinodermata—are composed primarily of radially or biradially symmetrical animals. These animals move slowly or not at all.

Bilateral symmetry is a common characteristic of animals that move freely through their environments. A bilaterally symmetrical animal can be divided into mirror images (left and right sides) by only a single plane that passes through the midline of its body from the front (anterior) to the back (posterior) end (Figure 31.3b). A plane at right angles to the first divides the body into two dissimilar sides; the side of a bilaterally symmetrical animal without a mouth is its dorsal (back) surface; the side with a mouth is its ventral (belly) surface.

Bilateral symmetry is strongly correlated with cephalization: the presence of a head, bearing sensory organs and central nervous tissues, at the anterior end of the animal. Cephalization may have been evolutionary advantageous because the anterior end of a freely moving animal typically encounters new environments first.

Speed is often advantageous for both prey and the predators that pursue them. Fast-moving prey and predators had evolved by the early Cambrian period. To move rapidly, an animal needs some type of skeleton that supports its body and allows body parts to be moved relative to one another. A skeleton may be internal or external, rigid or flexible, and be composed of one, two, or more elements.

The fluid-filled body cavities of early animals functioned as hydrostatic skeletons. Because fluids are relatively incompressible, they move to another part of the cavity when muscles surrounding them contract. If the body tissues around the cavity are flexible, fluids moving from one region cause some other region to expand. Moving fluids can thus move specific body parts, or even the whole animal, provided that temporary attachments can be made to the substrate.

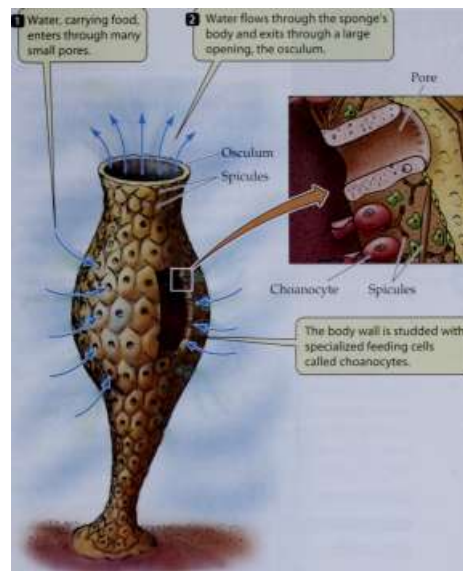
Other forms of skeletons developed in many animal lineages, either as substitutes for, or in combination with, hydrostatic skeletons. Some of these skeletons consist of a single element (snail shells); some have two elements (clam shells); others have many elements (centipedes). Some are internal (vertebrate bones); others are external (crab shells). The form of the body cavity also changed in many animal lineages. Many became divided into compartments. The form of its skeleton and body cavities strongly influences the degree to which an animal can control and change its shape, and thus the complexity of the movements it can perform. What type of body plan and symmetry did the common ancestors of all animals possess? We are not certain, but because evidence suggests that animals evolved from colonies of flagellated cells, they may have been similar in structure to living flagellates.

Sponges: Loosely Organized Animals

The difference between protist colonies and simple multicellular animals is that the cells of animals are differentiated and their activities are coordinated. The lineage leading to modern sponges separated from the lineage leading to all other animals early during animal evolution. Some living

I Water, carrying food, enters through many small pores.

Water flows through the sponge's body and exits through a large opening, the osculum.



ANIMAL ORIGINS AND LOPHOTROCHOZOANS 547

The sponges (phylum Porifera, Latin for "pore bearers") are sessile. They live attached to the sub-strate

Cnidaria



and do not move about. The body plan of all sponges— even large ones, which may reach more than a meter in



Ctenophora

Lophotrochozoans

PROTOSTOMES

Ecdysozoans

DEUTEROSTOMES

The body wall is studded specialized feeding cells called choanocytes.

37.4 The Sponge Body Plan

The flow of water through the sponge is shown by blue arrows. The body wall is studded with choanocytes, a type of specialized feeding cell that may be a link between animals and protists (see Figure 27.28).

sponges are still very similar to the probable ancestral colonial protists. Sponges are loosely organized. Even if a sponge is completely disassociated by being strained through a filter, its cells can reassociate into a new sponge.

length—is an aggregation of cells built around a water canal system. A sponge feeds by drawing water into itself and filtering out the small organisms and nutrient particles that flow past the walls of its inner cavity.

Feeding cells with a collar and a flagellum, called choanocytes, line the inside of the water canals. By beating their flagella, the choanocytes cause water to flow into the animal, either by way of small pores that perforate special epidermal cells (in simple sponges) or through intercellular pores (in complex sponges). Water passes into small chambers within the body where food particles are captured by the choanocytes. Water then exits through one or more larger openings called oscula (Figure 31.4).

Between the thin epidermis and the choanocytes is a layer of cells, some of which are similar to amoebas and move about within the body. A supporting skeleton is also present, either in the form of simple or branching spines called spicules or as an elastic, often complex, network of fibers.

Most of the 10,000 species of sponges are marine animals; only about 50 species live in fresh water. Sponges come in a wide variety of sizes and shapes that are adapted to different patterns of water movement (Figure 31.5). Sponges living in intertidal or shallow subtidal environ-

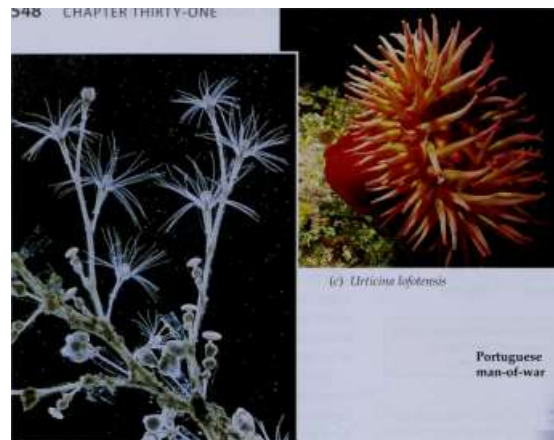


(a) *Euplectella aspergillum* (b) *Clathrina coriacea*

31.5 Sponges Differ in Size and Shape

(a) Glass sponges are named after their glasslike spicules, which are formed of silicon. (b) The spicules of this marine sponge are made of calcium carbonate, (c) The brown volcano sponge is typical of many simple marine sponges.

548 CHAPTER THIRTY-ONE



merits, where they are subjected to strong wave action, hug the substrate. Many sponges that live in calm waters are simple, with a single large opening on top of the body. Most sponges that live in flowing water are flattened and are oriented at right angles to the direction of current flow; they intercept water and the prey it contains as it flows past them.

Sponges reproduce both sexually and asexually. In most species, a single individual produces both eggs

Portuguese man-of-war

(a) *Gonofhyraea loveni*

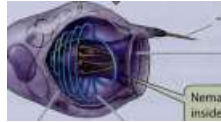


Cnidocytes

Cnidocyte before nematocyst discharge



(



Trigger"

Operculum (door)

Chrysaora fuscescens

31.6 Diversity among Cnidarians

(a) The structure of the polyps on a North Atlantic coastal hydrozoan is visible here, (b) This sea nettle jellyfish illustrates the complexity of some scyphozoan medusae. (c) The nematocyst-studded tentacles of this white-spotted anemone from British Columbia are poised to capture large prey carried to the animal by water movement.

Nematocyst capsule

After discharge

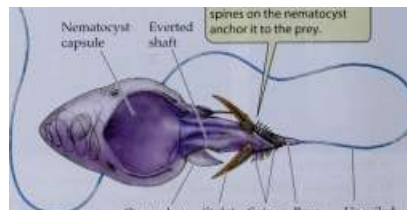
Coiled

nematocyst

tube

Nematocysts remain coiled inside cnidocytes until their discharge is triggered by the presence of potential prey.

Once discharged, stylets and spines on the nematocyst anchor it to the prey.



31.7 Nematocysts Are Potent Weapons

Possessing a large number of nematocysts, cnidarians such as jellyfish can subdue and eat very large prey.

Operculum

Stylet (barb)

Spines Base

of tube

Uncoiled

nematocyst

tube

and sperm. Water currents carry sperm from one individual to another. Asexual reproduction is by budding and fragmentation.

Cnidarians: Cell Layers and Blind Guts

Animals in all phyla other than the Porifera have distinct cell layers and symmetrical bodies. The next lineage to split off from the main line of animal evolution after the sponges resulted in a phylum of animals—the cnidarians (phylum Cnidaria) — having only two cell layers (diploblastic) and a blind gut (with only one entrance). Within the constraints of this simple body organization, cnidarians evolved a wide variety of ways of making a living.

Cnidarians are simple but specialized carnivores

Cnidarians (phylum Cnidaria) appeared early in evolutionary history and radiated in the late Precambrian. About 10,000 cnidarian species—jelly fishes, sea anemones, corals, and hydrozoans—are living today (Figure 31.6). All but a few are marine. The smallest cnidarians can hardly be seen without a microscope; the largest known jellyfish is 2.5 meters in diameter. These animals are simple but specialized carnivores. The cnidarian body plan combines a low metabolic rate with the ability to capture large prey. These traits allow cnidarians to survive in environments where prey are scarce.

A key feature of cnidarians is tentacles that bear cnidocytes, specialized cells that contain stinging structures called nematocysts that can discharge toxins into their prey (Figure

Porifera

Ctenophora

Lophotrochozoans

PROTOSTOMES

Ecdysozoans

DEUTEROSTOMES

31.7). Cnidocytes allow cnidarians to capture prey larger and more complex than themselves. Nematocysts are responsible for the sting that some jellyfishes and other cnidarians can inflict on human swimmers.

The mouth of a cnidarian is connected to a blind sac called the gastrovascular cavity, which functions in digestion, circulation, and gas exchange. The single opening serves as both mouth and anus. Cnidarians also have epithelial cells with muscle fibers whose contractions enable the animals to move, as well as nerve nets that integrate their body activities.



Cnidarian life cycles

The generalized cnidarian life cycle has two distinct stages, the polyp and the medusa (Figure 31.8), although many species lack one of the stages.

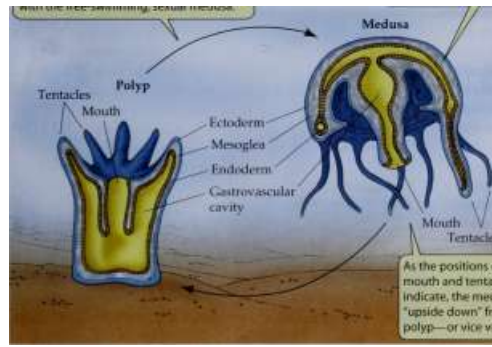
► The sessile polyp stage has a cylindrical stalk attached to the substrate, with tentacles surrounding a mouth located at the opposite end from the site of attachment. This stage is usually asexual, but individual polyps may reproduce by budding, thereby forming a colony.

► The medusa is a free-swimming, sexual stage shaped like a bell or an umbrella. It typically floats with its mouth and tentacles facing downward. Medusae produce eggs and sperm and release them into the water. When an egg is fertilized, it develops into a free-swimming, ciliated larva called a planula that eventually settles to the bottom and transforms into a polyp.

Although the polyp and medusa stages appear very different, they share a similar body plan. A medusa is essentially a polyp without a stalk. Most of the outward differences between polyps and medusae are due to the mesoglea, a mass of jellylike material that lies between the two cell layers. The mesoglea contains few cells and has a low metabolic rate. In polyps, the mesoglea is usually thin; in medusae it is very thick, constituting the bulk of the animal.

During the life cycle of many cnidarians, the usually sessile, asexual polyp alternates with the free-swimming, sexual medusa.

The mesoglea is a jellylike layer with few cells.



As the positions of the mouth and tentacles indicate, the medusa is "upside down" from the polyp—or vice versa.

hydrozoans. Life cycles are diverse among the hydrozoans (class Hydrozoa), a group containing the only freshwater cnidarians. The polyp commonly dominates the life cycle, but some species have only medusae and others only polyps. A few species have solitary polyps, but most hydrozoans are colonial. A single planula eventually gives rise to a colony of many polyps, all interconnected and sharing a continuous gastrovascular cavity (Figure 31.9). Within such a colony, some polyps have tentacles with many nematocysts; they capture prey for the colony. Others lack ten-

31.8 A Generalized Cnidarian Life Cycle

Cnidarians typically have two body forms, one asexual (the polyp) and the other sexual (the medusa).

550 CHAPTER THIRTY-ONE

31.9 Hydrozoans Often Have Colonial Polyps

The polyps with a hydrozoan colony may differentiate to perform specialized tasks.

an

Medusae develop within enlarged polyp.

If The polyps of the hydrozoan *Obelia* are interconnected and share gastrovascular cavities.

tacles and are unable to feed, but are specialized for the production of medusae. Still others are fingerlike and defend the colony.

scyphozoans. The several hundred species of the class Scyphozoa are all marine. The mesoglea of their medusae is very thick and firm, giving rise to their common name, jelly fishes. The medusa typically has the form of an inverted cup, and the tentacles with nematocysts extend downward from the margin of the cup.

The medusa, rather than the polyp, dominates the life cycle of scyphozoans. An individual medusa is male or female, releasing eggs or sperm into the open sea. The fertilized egg develops into a small planula that quickly settles on a substrate and changes into a small polyp. This polyp feeds and grows and may produce additional polyps by budding. After a period of growth, the polyp begins to bud off small medusae (Figure 31.10). These small medusae feed, grow, and transform themselves into adult medusae, which are commonly seen during summer in harbors and bays. Thus a polyp that grows from a single fertilized egg is capable of producing many genetically identical medusae that will eventually reproduce sexually.

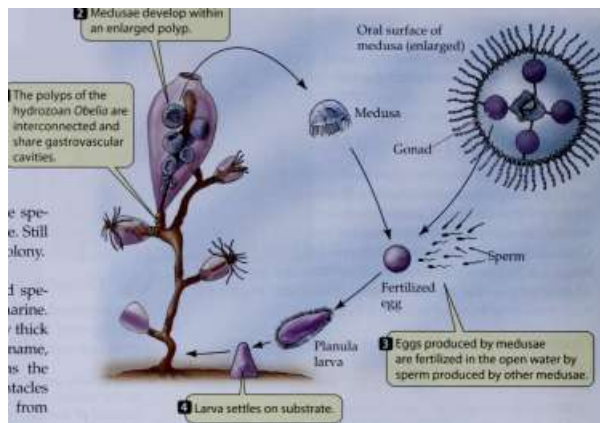
anthozoans. The roughly 6,000 species of sea anemones and corals that constitute the anthozoans (class Anthozoa) are all marine. Unlike other cnidarians, anthozoans entirely lack the medusa stage of the life cycle. The polyp produces eggs and sperm, and the fertilized egg develops into a planula that develops directly into another polyp. Many species can also reproduce asexually by budding or fission.

Sea anemones (see Figure 31.6c) are solitary. They are widespread in both warm and cold ocean waters. Many sea anemones are able to crawl slowly on the discs with which they attach themselves to the substrate. A few species can swim; some can burrow.

Corals, by contrast, are usually sessile and colonial. The polyps of most corals secrete a matrix of organic molecules upon which calcium carbonate—the eventual skeleton of the coral colony—is deposited. The forms of

31.10 Medusae Dominate Scyphozoan Life Cycles

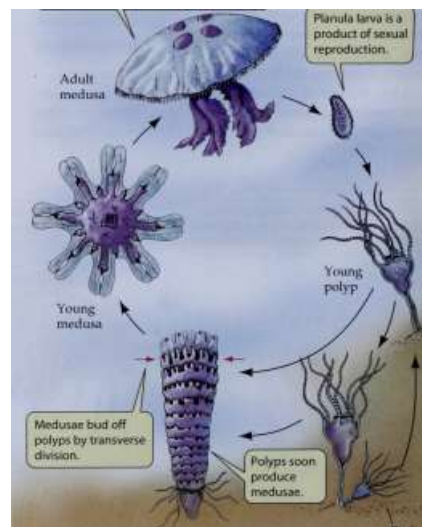
Scyphozoan medusae are the familiar jellyfish of coastal waters. The small, sessile polyps quickly produce medusae.



are fertilized in the open water by sperm produced by other medusae.

Scyphozoan medusae are the dominant life form and are familiar to us as jellyfish.

Planula larva is a product of sexual reproduction.



Bud

ANIMAL ORIGINS AND LOPHOTROCHOZOANS



(a)

31.11 Corals

(a) Many different species of corals and sponges grow together on this reef in the Bahama Islands. (fc>) The green plates of cabbage coral (*Turbinaria* sp.) and the branching staghorn coral (*Acrophora* sp.) are oriented to intercept sunlight in this Papua New Guinea reef.

coral skeletons are species-specific and highly diverse (Figure 31.11a). The common names of coral groups—horn corals, brain corals, staghorn corals, organ pipe corals, sea fans, and sea whips, among others—describe their appearance.

As a coral colony grows, old polyps die, but their calcareous skeletons remain. The living members form a layer on top of a growing reef of skeletal remains, eventually forming chains of islands and reefs. Corals are especially abundant in the Indo-

Pacific region. The Great Barrier Reef along the northeastern coast of Australia is a system of coral formations more than 2,000 km long and as wide as 150 km. A continuous coral reef hundreds of kilometers long in the Red Sea has been calculated to contain more material than all the buildings in the major cities of North America combined.

Corals flourish in nutrient-poor, clear, tropical waters. For a long time scientists wondered how corals obtain enough nutrients to grow rapidly. The answer is that photo-synthetic dinoflagellates live symbiotically within a coral's cells. They provide the corals with products of photosynthesis and contribute to calcium deposition. In turn, the corals protect the dinoflagellates from predators. This symbiotic relationship explains why reef-forming corals are restricted to clear surface waters, where light levels are high enough to allow photosynthesis (Figure 31.11).

Coral reefs throughout the world are being threatened by both global warming, which is raising the temperatures of tropical shallow ocean waters, and nutrient runoff from developments on adjacent shorelines. An overabundance of

(b)

nitrogen gives an advantage to algae, which overgrow the corals and smother them.

Ctenophores: Complete Guts and Tentacles

Ctenophores (phylum Ctenophora) were the next lineage to separate from the lineage leading to all other animals. Ctenophores, also known as comb jellies, have body plans that are superficially similar to those of cnidarians. Both have two cell layers separated by a thick, gelatinous meso-glea, and both have radial symmetry and feeding tentacles. Like cnidarians, ctenophores have low metabolic rates because they are composed primarily of inert mesoglea. Unlike cnidarians, however, ctenophores have a complete gut. Food enters through a mouth and wastes are voided through two anal pores.

Ctenophores have eight comblike rows of fused plates of cilia, called combs. They move by beating these Cnidaria

cilia rather than by use of muscular contractions. Ctenophoran Lophotrochozoans , . , PROTOSTOMES

tentacles do not have nematocysts

nematocysts; rather, they are covered with sticky filaments to which prey adhere (Figure 31.12). After capturing its prey, a ctenophore retracts its tentacles to bring the food to its mouth. In some species, the entire surface of the body is coated with a sticky mucus that captures prey. All of the 100 known species of ctenophores are marine carnivores. They are common in open seas, where prey are often scarce. Most ctenophores cannot capture large prey.

Ctenophore life cycles are simple. Gametes from gonads located on the walls of the gastrovascular cavity are released into the cavity and then discharged through the

Porifera

DEUTEROSTOMES

552 CHAPTER THIRTY-ONE

(b) Leucothea sp.

(a)

Anal

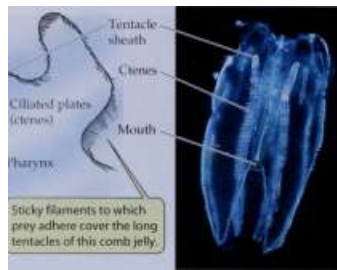
Gut

Tentacle

Tentacles lack nematocysts.



Ciliated plates (ctenes



Pharynx

Mouth

Sticky filaments to which prey adhere cover the long tentacles of this comb jelly.

37.72 Comb Jellies Feed with Tentacles

(a) The body plan of a typical ctenophore. (fa) This comb jelly has much shorter tentacles than many other ctenophores.

mouth or through pores. Fertilization takes place in the open seawater. In nearly all species, the fertilized egg develops directly into a miniature ctenophore that gradually grows into an adult.

The Evolution of Bilaterally Symmetrical Animals

The phylogenetic tree in Figure 31.2 postulates a common ancestor of all bilaterally symmetrical animals, but it does not tell us what that common ancestor looked like. Evolutionary biologists have attempted to infer the nature of those ancestral animals, which they call urbilateria, using evidence from the genes, development, and structure of existing animals.

One clue is provided by the fact that all living bilaterally symmetrical animals have an array of intercellular signaling systems and many homeobox gene families (see Chapter 16). The simplest bilaterally symmetrical animals have only a few homeobox genes, but some of them are shared with more complex animals. The mechanisms that regulate embryonic development in the protostomes and deuterostomes are governed by homologous homeobox genes. Such regulatory genes with similar functions are unlikely to have evolved independently in several different animal lineages.

Some evidence that urbilaterians may have been relatively complex is provided by fossilized traces of their movements. Fossilized trails from late Precambrian times exhibit complex search patterns, transverse furrows, and longitudinal ridges (Figure 31.13). They were made by organisms that were at least several centimeters long. The complexity of the movements recorded by the tracks suggests that urbilaterians had circulatory systems, systems of antagonistic muscles, and a tissue or fluid-filled body cavity. Some of their descendants subsequently lost some of those traits, but they retain signatures of their past in their genes.

Protostomes and

Deuterostomes:

An Early Lineage Split

The next major lineage split in the evolution of animals separated two groups that have been evolving separately ever since the Cambrian period. These two major lineages—the protostomes and deuterostomes—dominate today's biota. Members of both lineages are bilaterally symmetrical and have definite heads (cephalization). Because their skeletons and body cavities are more complex than those of the animals we have discussed so far, they are capable of more elaborate movements.

The most important shared, derived traits that unite the protostomes are a

. Porifera

central nervous

System consist Cnidaria

ing of an anterior brain that surrounds the entrance to the digestive tract; a ventral nervous system consisting of paired or fused longitudinal nerve cords; and a free-floating larva with a food-collecting system consisting of compound cilia on multiciliate cells. The major shared, derived traits that unite the deuterostomes are a dorsal nervous system and larvae with a food-collecting system consisting of cells with a single cilium.

Ctenophora



DEUTEROSTOMES



Hiemalora

1 cm

37.73 Fossilized Trail of an Urbilaterian

These tracks indicate that their maker was able to crawl.

37.74 A Phylogeny of Lophotrochozoans

Three major lineages, including the lophophorate and spiralian phyla, dominate the tree. Some small phyla are not included in this diagram.

Three cell layers

Lopho-trochozoan ■ (ancestor

Most of the world's living animal species are protostomes. The diversity of protostome body plans and lifestyles has posed many challenges to zoologists attempting to infer the evolutionary relationships among these animals. Developmental, structural, and molecular data all suggest that protostomes split into two major lineages that have been evolving independently since ancient times: the lophotrochozoans and the ecdysozoans.

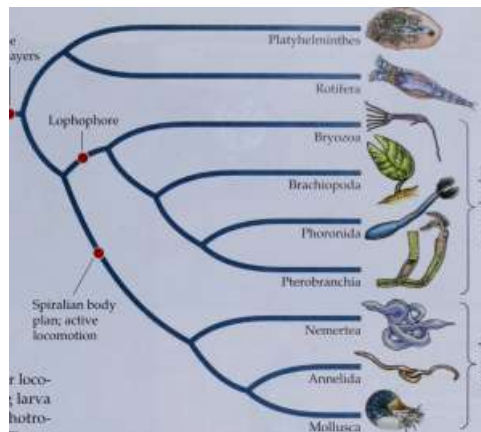
Lophotrochozoans, the animals we will discuss in the remainder of this chapter, grow by adding to the size of their skeletal elements. They use cilia for locomotion, and many lineages have a type of free-living larva known as a trochophore. The phylogeny of lophotrochozoans we will use in this chapter is shown in Figure 31.14. In contrast, ecdysozoans, the animals we will discuss in the next chapter, increase in size by molting their external skeletons. They move by mechanisms other than ciliary action, and they share a common set of homeobox genes.

Simple Lophotrochozoans

Flatworms move by beating cilia

Members of the phylum Platyhelminthes, or flatworms, are the simplest lopho-

trochozoans (Figure 31.15). They are bilaterally symmetrical animals that have no enclosed body cavity. They lack organs for transporting oxygen to internal tissues, and they have only simple organs for excreting metabolic wastes. This body plan dictates that each cell must be near a body surface, a requirement met by the flattened body form.



Spiralian body plan; active locomotion

Platvhelminthes

Rotifera

Bryozoa

Brachiopoda

Phoronida

Pterobranchia

Nemertea

Annelida

Mollusca

Mollusca

The digestive tract of a flatworm consists of a mouth opening into a blind sac. However, the sac is often highly branched, forming intricate patterns that increase the surface area available for absorption of nutrients. All living flatworms feed on animal tissues—living or dead. Motile flatworms glide over surfaces, powered by broad bands of cilia. This form of movement is very slow, but it is sufficient

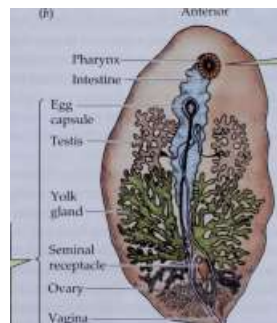
37.75 Flatworms Live Freely and Parasitically

(a) Some flatworm species are free-living, like this marine flatworm of the South Pacific, (b) The flatworm diagrammed here lives parasitically in the gut of sea urchins. It is representative of parasitic flukes. Because their hosts provide all the nutrition they need, intestinal parasites do not require elaborate feeding or digestive organs.

Anterior



As is typical of internal parasites, the flatworm's body is filled primarily with sex organs.



The flatworm gut has a single exterior opening. The pharynx is both "mouth" and "anus."

Posterior

554 CHAPTER THIRTY-ONE

tThe fish is eaten by a mammalian host; tapeworm matures.



t

The perch is eaten by a larger fish

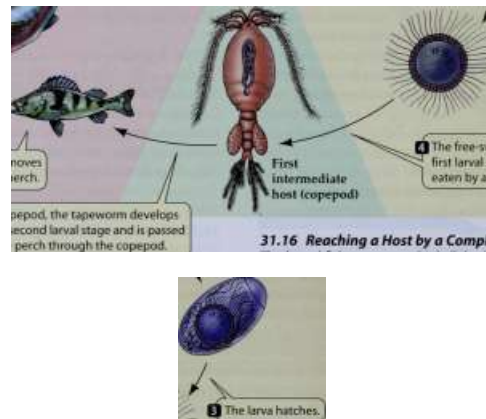


f

Second ^^

intermediate host (fish)

The third larval stage moves to the muscles of the perch.



The free-swimming first larval stage is eaten by a copepod.

| In the copepod, the tapeworm develops into the second larval stage and is passed on to the perch through the copepod.

for small, scavenging animals. Parasitic species that absorb digested food from their hosts do not have a digestive tract.

The flatworms probably most similar to the ancestral forms are the turbellarians (class Turbellaria), which are small, free-living marine and freshwater animals (a few live in moist terrestrial habitats). Freshwater turbellarians of the genus *Dugesia*, better known as planarians, are the most familiar species of flatworms. At one end they have a head with chemoreceptor organs, two simple eyes, and a tiny brain composed of anterior thickenings of the longitudinal nerve cords.

Although the earliest flatworms were free-living (Figure 31.15f), many species evolved a parasitic existence. A likely evolutionary transition was from feeding on dead organisms, to feeding on the body surfaces of dying hosts, to invading and consuming parts of living, healthy hosts. Most of the 25,000 species of living flatworms—including the tapeworms (class Cestoda) and flukes (class Trematoda; Figure 31.15b)—are parasitic. These worms inhabit the bodies of many other species, including vertebrates; some cause serious human diseases. Monogeneans (class Mono-

37.76 Reaching a Host by a Complex Route

The broad fish tapeworm *Diphyllobothrium latum* must pass through the bodies of a copepod (a type of crustacean) and a fish before it can reinfect its primary host, a mammal. Such complex life cycles assist the flatworm's recolonization of hosts, but they also offer opportunities for humans to break the cycle with hygienic measures.

genea) are external parasites of fishes and other aquatic vertebrates.

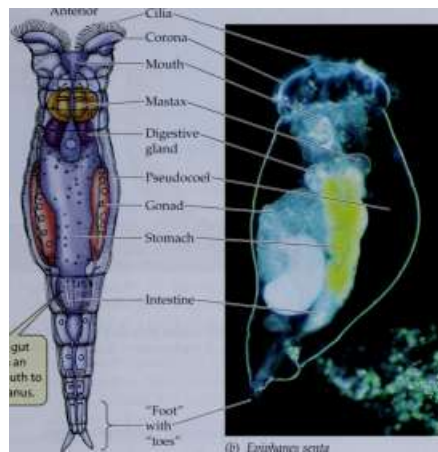
Parasitic flatworms live in nutrient-rich environments in which food is delivered to them, but they face other challenges. To complete their life cycle, parasites must overcome the defenses of their host. And because they die when their host dies, their offspring must disperse to new hosts. The eggs of some parasitic flatworms are voided with the host's feces and later ingested directly by other host individuals. However, most parasitic species have complex life cycles involving two or more hosts and several larval stages (Figure 31.16).

Rotifers are small but structurally complex

Rotifers (phylum Rotifera) are bilaterally symmetrical, pseudocoelomate, unsegmented animals that have three cell

Anterior

A complete gut passes from an anterior mouth to a posterior anus.



Posterior

(b) *Epiphanes senta*

{a} *Philadina roseola*

31.17 Rotifers

(a) This rotifer reflects the general structure of many free-living species in this phylum. (fc>) The internal anatomy of a rotifer is clear in this micrograph.

layers. Most rotifers are tiny (50-500 μm long)—smaller than some ciliate protists—but they have highly developed internal organs (Figure 31.17). A complete gut passes from an anterior mouth to a posterior anus; the pseudocoel functions as a hydrostatic skeleton. Most rotifers propel themselves through the water by means of rapidly beating cilia rather than by muscular contraction. This type of movement is effective because rotifers are so small.

The most distinctive organs of rotifers are those used to collect and process food. A conspicuous ciliated organ called the corona surmounts the head of many species. Coordinated beating of the cilia provides the force for locomotion and also sweeps particles of organic matter from the water into the mouth and down to a complex structure (the mastax) where the food is ground. By contracting the muscles that surround the pseudocoel, a few rotifer species that prey on protists and small animals can protrude the mastax through the mouth and seize small objects with it.



Platyhelminthes

Bryozoa Brachiopoda

Phoronida

Pterobranchia

Nemertea

Annelida

Mollusca

Some rotifers are marine, but most of the 1,800 known species live in fresh water. Members of a few species rest on the surface of mosses and lichens in a desiccated, inactive state until it rains. When rain falls, they absorb water and become motile, feeding in the films of water that temporarily cover the plants. Most rotifers live no longer than 1 or 2 weeks.

Lophophorates: An Ancient Body Plan

After the platyhelminthes and rotifers diverged from it, the lophotrochozoan lineage divided into two branches. The descendants of these branches became the lophophorates—the subject of this section—and the spiralian, which we will describe in the following section.

Four phyla of lophophorate animals survive today: Phoronida, Brachiopoda, Ecto-procta, and Pterobranchia. Nearly all members of these phyla are marine; only a few species live in fresh water. About 4,500 living species are known, but many times that number existed during the Paleozoic and Mesozoic eras. These animals all have a body divided into three parts: prosome (anterior), mesosome (middle), and metasome (posterior). In most species, each region has a separate coelomic compartment: the protoel, mesocoel, and metacoel, respectively.

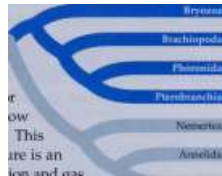
Lophophorate animals obtain food by filtering it from ocean waters, a trait that they shared with many other protostomes.

The most conspicuous feature of these animals, the lo-phophore, is a circular or U-shaped ridge around the mouth that bears one or two rows of ciliated, hollow tentacles (Figure 31.18). This large and complex structure is an organ for both food collection and gas exchange. All adult lophophorate animals are sessile; they use the tentacles and cilia of the lophophore to capture plankton. Lophophorates also have a U-shaped gut; the anus is located close to the mouth, but outside the tentacles.

The 20 known species of phoronids (phylum Phoronida) are sedentary worms that live in muddy or sandy sediments or attached to a rocky substrate. Phoronids are found in waters ranging from intertidal zones to about 400 meters deep. They range in size from 5 to 25 cm in length, and they secrete chitinous tubes in which they live. The lophophore is the most conspicuous external feature of

Platyhelminthes

Rotifera



Nemertea

Annelida

Mollusca

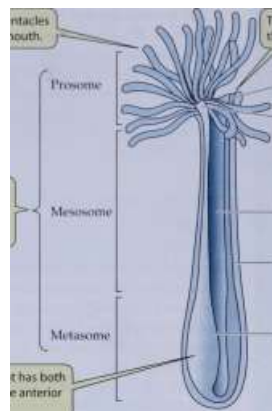
556 CHAPTER THIRTY-ONE

Anterior

Lophophore tentacles surround the mouth.

Lophophorates have a body plan that is divided into three parts.

A U-shaped gut has both openings at the anterior of the animal.



The anus is outside the ring of tentacles.

Arms

M

Tentacles

Anus

Mouth

lube

Stalk

Metacoel

Posterior

37.78 Lophophore Artistry

The lophophore dominates the anatomy of a phoronid. The phoronid gut is U-shaped.

phoronids (see Figure 31.18). Cilia drive water into the top of the lophophore. Water exits through the narrow spaces between the tentacles. Suspended food particles are caught and transported by ciliary action to the food groove and into the mouth.

There are only 10 living species of pterobranchs (phylum Pterobranchia). Pterobranchs are sedentary animals up to 12 mm in length that live in tubes secreted by a proboscis, which is homologous to the prosome of phoronids. Some species are solitary; others form colonies of individuals joined together (Figure 31.19). Behind the proboscis is a collar with 1-9 pairs of arms bearing long tentacles that capture prey and permit gas exchange.

(a) *Iodyticium* sp.

(b)



31.19 Pterobranchs May Be Colonial or Solitary

This drawing of *Rhabdopleura* depicts two members of a colony.

Bryozoans Are Colonial Lophophorates

Bryozoans (phylum Ectoprocta) are colonial lophophorates that live in a "house" secreted by the body wall. A colony consists of many small individuals connected by strands of tissue along which materials can be moved (Figure 31.20f). Most bryozoans are marine, but a few live in fresh water. They are able to completely retract the lophophore, which they can also rock and rotate to increase contact with prey (Figure 31.20b).

A colony of bryozoans is created by the asexual reproduction of its founding members. One colony may contain as many as 2 million individuals. In some species, individual colony members are specialized for feeding, reproduction, defense, or support. Bryozoans reproduce sexually by releasing sperm into the water, where they are collected by other individuals. Eggs are fertilized internally, and devel-

31.20 Bryozoans

(a) Branching colonies of bryozoans may appear plantlike. (6) Bryozoans have greater control over the movement of their lophophores than members of other lophophorate phyla.

Bryozoans use various muscles to extend and retract their lophophores.

They can also rock and rotate the lophophore to increase contact with prey.



Lophophore Lophophore Lophophore Lophophore

extends spreads rocks and rotates retracts

oping embryos are brooded before they exit as larvae to seek suitable sites for attachment.

Brachiopods Superficially Resemble Bivalve Mollusks

Brachiopods (phylum Brachiopoda) are solitary, marine lophophorate animals that superficially resemble bivalve mollusks (Figure 31.21). Most brachiopods are between 4 and 6 cm long, but some are as long as 9 cm. Brachiopods have a shell divided into two parts connected by a ligament. The two halves can be pulled shut to protect the soft body. The shell differs from that of mollusks in that the two halves are dorsal and ventral rather than lateral. The two-armed lophophore of a brachiopod is located within the shell. The beating of cilia on the lophophore draws water into the slightly opened shell. Food is trapped in the lophophore and directed to a ridge along which it is transferred to the mouth.

Brachiopods are either attached to a solid substrate or embedded in soft sediments. Most species are attached by means of a short, flexible stalk that holds the animal above the substrate. Gases are exchanged across body surfaces, especially the tentacles of the lophophore. Most brachiopods release their gametes into the water, where they are fertilized. The larvae remain in the plankton for only a few days before they settle and change into adults.

Brachiopods reached their peak abundance and diversity in Paleozoic and Mesozoic times. More than 26,000 fossil species have been described. Only about 350 species survive, but they are common in some marine environments.

Lophophore



Laqueus sp.

37.27 Brachiopods

You can see the lophophore of this North Pacific brachiopod between the valves of its shell.

cles to move on the surface of sediments or to burrow. Movement by both of these methods is slow.

Within the body of almost all 900 species of ribbon worms is a fluid-filled cavity called the rhynchocoel, within which floats a hollow, muscular proboscis. The proboscis, which is the feeding organ, may extend much of the length of the worm. Contraction of the muscles surrounding the rhynchocoel causes the proboscis to be everted explosively through an anterior opening (Figure 31.22) without moving

Spiralians: Wormlike Body Plans

The spiralian lineage gave rise to many phyla. Members of more than a dozen of these phyla are wormlike; that is, they are bilaterally symmetrical, legless, soft-bodied, and at least several times longer than they are wide. This body form enables animals to move efficiently through muddy and sandy marine sediments. Most of these phyla have no more than several hundred species, even though the lineages have been evolving independently since early animal evolution.

The carnivorous ribbon worms (phylum Nemertea) are dorsoventrally flattened and have nervous and excretory systems similar to those of flatworms but, unlike flatworms, they have a complete digestive tract with a mouth at one end and an anus at the other. Food moves in one direction through the digestive tract of a ribbon worm and is acted on by a series of digestive enzymes. Small ribbon worms move by beating their cilia. Larger ones employ waves of contraction of body mus

oo

Platyhelminthes

Rotifera

Bryozoa

Brachiopoda

Phoronida

Pterobranchia



Floating in a cavity called the rhynchocoel, the proboscis can be moved rapidly. The worm, however, moves slowly.

Proboscis' pore



Mouth

Intestine Retractor muscle

Proboscis retractor muscle

Anus



Everted proboscis

(b)

31.22 Ribbon Worms

(a) The proboscis is the ribbon worm's feeding organ, (b) The full length of this ribbon worm from Oregon is impressive.



Tubulanus polymorphic

558 CHAPTER THIRTY-ONE

the rest of the animal. The proboscis of most ribbon worms is armed with a sharp stylet that pierces the prey. Paralysis-causing toxins produced by the proboscis are discharged into the wound made by the stylet.

Segmented Bodies: Improved Locomotion

A body cavity divided into segments allows an animal to alter the shape of its body in complex ways and to control its movements precisely. Fossils of segmented worms are known from the middle Cambrian; the earliest forms are thought to have been burrowing marine animals. Segmentation evolved several times among spiralian.

Annelids have many-segmented bodies

The annelids (phylum Annelida) are a diverse group of segmented worms (Figure 31.23). The approximately 15,000 known annelid species live in marine, freshwater, and terrestrial environments. A separate nerve center called a ganglion controls each segment, but the ganglia are connected by nerve cords that coordinate their functioning. The coelom in each segment is isolated from those in other segments. Most annelids lack a rigid, external protective covering. The thin body wall serves as a surface for gas exchange in most species, but this thin, permeable body surface restricts annelids to moist environments; they lose body water rapidly in dry air.

polychaetes. More than half of all annelid species are members of the class Polychaeta. Nearly all polychaetes are marine animals. Most have one or more pairs of eyes and one or more pairs of tentacles at the anterior end of the body. The body wall in most segments extends laterally as

a series of thin outgrowths, called parapodia, that contain many blood vessels. The parapodia function in gas exchange, and some species use them to move. Stiff bristles called setae protrude from each parapodium, forming temporary attachments to the substrate and preventing the animal from slipping backward when its muscles contract.

Many polychaete species live in burrows in soft sediments and filter prey from the surrounding water with elaborate feathery tentacles (Figure 31.24/?). Typically, males and females release gametes into the water, where the eggs are fertilized and develop into a trochophore larva. As the larva develops, it forms body segments at its posterior end, eventually changing into a small adult worm.

Platyhelminthes Rotifera Bryozoa Brachiopoda Phoronida



Pterobranchia

Mollusca

Ganglion in ventral nerve

Brain

Hearts

Circular muscle -

OLIGOCHAETES. More than 90 percent of the approximately 3,000 described species of oligochaetes (class Oligochaeta) live in freshwater or terrestrial habitats. Oligochaetes have no parapodia, eyes, or anterior tentacles, and they have relatively few setae. Earthworms—the most familiar oligochaetes— are scavengers and ingesters of soil, from which they extract food particles.

Unlike polychaetes, all oligochaetes are hermaphroditic: Each individual is both male and female. Sperm are exchanged simultaneously between two copulating individuals (Figure 31.24b). Eggs are laid in a cocoon outside the adult's body. The cocoon is shed, and when development is complete, miniature worms emerge and begin independent life.

Sperm receptacles Testes and sperm sacs Ovary

Oviduct Sperm duct

Longitudinal muscle

- Blood vessel ^ — Coelom

■ Intestine

Pairs of bristles

Blood vessels



Segments

Coelom

Excretory organs

leeches. Leeches (class Hirudinea) probably evolved from oligochaete ancestors. Most species live in freshwater or terrestrial habitats and, like oligochaetes, lack parapodia and tentacles. Like oligochaetes, leeches are hermaphroditic. The coelom of leeches is not divided into compartments, and the coelomic space is largely filled with mesenchyme tissue. Groups of segments at each end of a leech are modified to form suckers, which serve as temporary anchors that aid in movement (Figure 31.24c). With its posterior sucker attached to a substrate, the leech extends its body by contracting its circular muscles. The anterior sucker is

Pairs of bristles

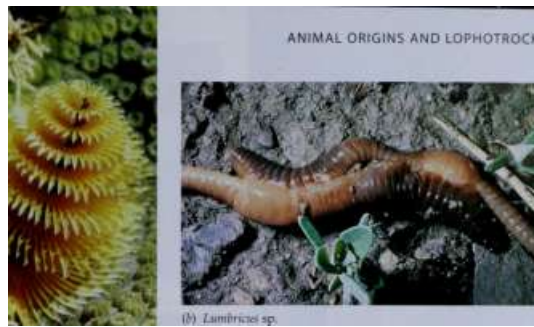
37.23 Many Body Segments

The segmented structure of the annelids is apparent both externally and internally. Most organs of this earthworm are repeated serially.

ANIMAL ORIGINS AND LOPHOTROCHOZOANS 559

% r

(fl) Spirobrnchniis sp.



(c) Australian tiger leech

3 1.24 Diversity among the Annelids

(a) The "feather cluster" worm is a marine annelid with striking feeding tentacles, (b) Individual earthworms are hermaphroditic (simultaneously both male and female). When they copulate, each individual both donates and receives sperm, (c) This Australian tiger leech is attached to a leaf by its posterior sucker as it waits for a mammalian "victim." (d) Vestimentiferans live around thermal vents deep in the ocean. Their skin secretes chitin and other substances, forming tubes from which they extend feeding tentacles.

then attached, the posterior one detached, and the leech shortens itself by contracting its longitudinal muscles.

Many leeches are external parasites of other animals, although some species eat snails and other invertebrates. A parasitic leech makes an incision in its host to expose its blood. It can ingest so much blood in a single feeding that its body may enlarge several times. A substance called hirudin secreted by the leech into the wound keeps the host's blood flowing (and gives this class its name; see the opening page of Chapter 6). For hundreds of years leeches were widely employed in medicine for bloodletting. Even today leeches are used to reduce fluid pressure and prevent blood clotting in damaged tissues and to eliminate pools of coagulated blood.

vestimentiferans. Members of one lineage of annelids, the vestimentiferans, evolved into burrowing forms with a crown of tentacles through which gases are exchanged, and entirely lost their digestive systems (Figure 31.24d). The coel-



om of a vestimentiferan consists of an anterior compartment, into which the tentacles can be withdrawn, and a long, subdivided cavity that extends much of the length of its body. Experiments using radioactively labeled molecules have shown that vestimentiferans take up dissolved organic matter at high rates v from either the sediments

k*^'^ in which they live or the

surrounding water.

Vestimentiferans were not discovered until the twentieth century, when deep-ocean exploration revealed them living many thousands of meters below the surface. In these deep oceanic sediments they are abundant, reaching densities of many thousands per square meter. About 145 species have been described. The largest and most remarkable vestimentiferans, which grow to 2 meters in length, live near deep-ocean hydrothermal vents—openings in the seafloor through which hot, sulfide-rich water pours. The tissues of these species harbor endosymbiotic prokaryotes that fix carbon using energy obtained from the oxidation of hydrogen sulfide (H_2S).

Mollusks lost segmentation but evolved shells

Mollusks (phylum Mollusca) range in size from snails only a millimeter high to giant squids more than 18 meters long—the largest known pro-tostomes. Beginning with a segmented common ancestor, mollusks underwent one of the most

Platyhelminthes

Rotifera

Bryozoa

Brachiopoda

Phoronida

Pterobranchia

Nemertea

Annelida

560 CHAPTER THIRTY-ONE

Generalized molluscan body plan

Shell Stomach Radula

Mouth



Digestive

gland

Intestine Mantle Anus

Mantle cavity

Heart

Foot

Chitons

In all mollusk lineages, a mantle covers the internal organs of the visceral mass.

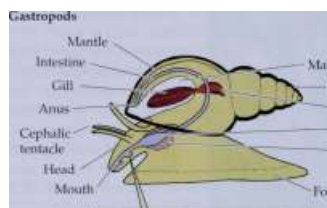


Intestine Stomach — /

~/c2?

Mouth Digestive gland

Foot Gills in mantle cavity



Cephalic tentacle

Head Mouth

Mantle cavity

Shell

Heart

- Stomach

Digestive gland

Foot

The radula is a unique molluscan feeding structure modified for scraping.



In bivalve mollusks, the foot is modified for burrowing.

Cephalopods

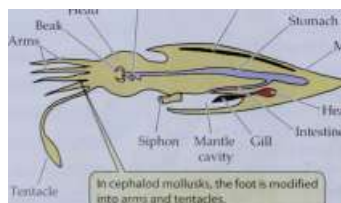
Head

Radula

Shell

Stomach

Mantle



Siphon Mantle Gill cavity

Heart Intestine

Tentacle

In cephalopod mollusks, the foot is modified into arms and tentacles.

dramatic of animal evolutionary radiations, based on a body plan with three major structural components: a foot, a mantle, and a visceral mass. Animals that appear very different, such as snails, clams, and squids, are all built from these three components (Figure 31.25).

► The molluscan foot is a large, muscular structure that originally was both an organ of locomotion and a support for the internal organs.

In the lineage leading to squids and octopuses, the foot was modified to form arms and tentacles borne on a head with complex sensory organs. In other groups, such as clams, the foot was transformed into a burrowing organ. In some lineages the foot is greatly reduced.

► The mantle is a fold of tissue that covers the visceral mass of internal organs. In many mollusks, the mantle extends beyond the visceral mass to form a mantle cavity.

The gills, which are used for gas exchange and, in some species, for feeding, lie in the mantle cavity. When the cilia on the gills beat, they create a flow of oxygenated water over the gills.

The coelom of mollusks is much reduced, but the open circulatory system has large fluid-filled cavities that are major components of a hydraulic skeleton.

The mollusks also developed a rasping feeding structure known as the radula. The radula was originally an organ for scraping algae from rocks, a function it retains in many living mollusks. However, in some mollusks, it has been modified into a drill or a poison dart. In others, such as clams, it is absent.

Although individual components have been lost in some lineages, these three unique shared derived characteristics are why zoologists believe that all 100,000 species of mollusks share a common ancestor. A small sample of these species is shown in Figure 31.26.

monoplacophorans. Monoplacophorans (class Mono-placophora) were the most abundant mollusks during the Cambrian period, but today there only a few surviving species. Unlike all other living mollusks, the surviving monoplacophorans have multiple gills, muscles, and excretory structures that are repeated over the length of the body. The gills are located in a large cavity under the shell, through which oxygen-bearing water circulates.

CHITONS. Chitons (class Polyplacophora) have multiple gills and segmented shells, but their other body parts are not segmented (Figure 31.26<?). The chiton body is bilaterally symmetrical, and its internal organs, particularly the digestive and nervous systems, are relatively simple. The

31.25 Molluscan Body Plans

The diverse modern mollusks are all variations on a general body plan that includes a foot, a mantle, and a visceral mass of internal organs.



(a) *Tonicella hneata*



(c) *Hypsclodoris* sp.



(e) *Octopus cyanea*

31.26 Diversity among the Mollusks

(a) Chitons are common in the intertidal zones of the North American coast, (b) The giant clam of Indonesia is among the largest of the bivalve mollusks. (c) Slugs are terrestrial and marine gastropods that have lost their shells; this shell-less sea slug is very conspicuously colored, (d) Land snails are shelled, terrestrial gastropods, (e) Cephalopods such as the octopus are active predators. (o) The boundaries of its chambers are clearly visible on the outer surface of this shelled Nautilus, another cephalopod.



(/) *Nautilus belavensis*



562 CHAPTER THIRTY-ONE

trochophore Larvae of chitons are almost indistinguishable from those of annelids. Most chitons are marine herbivores that scrape algae from rocks with their sharp radulae. An adult chiton spends most of its life glued tightly to rock surfaces by its large, muscular, mucus-covered foot. It moves slowly by means of rippling waves of muscular contraction in the foot

bivalves. One lineage of early mollusks developed a hinged, two-part shell that extended over the sides of the body as well as the top, giving rise to the bivalves (class Bivalvia), which include the familiar clams, oysters, scallops, and mussels (Figure 31.26b). Bivalves are largely sedentary and have greatly reduced heads. The foot is compressed and, in many clams, is used for burrowing into mud and sand. Bivalves feed by bringing water in through an opening called a siphon and extracting food from the water using their large gills, which are also the main sites of gas exchange. Water exits through another siphon.

gastropods. Another lineage of early mollusks gave rise to the gastropods (class Gastropoda), which includes the snails. Most gastropods are motile, using the large foot to move slowly across the substrate or to burrow through it. Gastropods are the most species-rich and widely distributed of the molluscan classes (Figure 31.26c,d). Some species, such as snails, whelks, limpets, slugs, abalones, and the often brilliantly ornamented nudibranchs, can crawl. Others—the sea butterflies and heteropods—have a modified foot that functions as a swimming organ with which they move through open ocean waters. The only mollusks that live in terrestrial environments—land snails and slugs—are gastropods. In these terrestrial species the mantle cavity is modified into a highly vascularized lung.

cephalopods. In one lineage of mollusks, the cephalopods (class Cephalopoda), the exit siphon, which initially may have simply improved the flow of water over the gills, became modified to allow the early cephalopods to control the water content of the mantle cavity. The modification of the mantle into a device for forcibly ejecting water from the cavity enabled cephalopods to move rapidly through the water. Furthermore, as fluid moves out of a chamber, gases diffuse into it, changing the buoyancy of the animal. Thus, by pumping out water, the animals could also control their buoyancy. Together, these adaptations allowed cephalopods to live in open water.

With their greatly enhanced mobility, some cephalopods, such as squids and octopuses, became the major predators in open ocean waters (Figure 31.26e). They are still important marine predators today. Cephalopods capture and subdue their prey with their tentacles; octopuses use theirs to move over the substrate. As is typical of active predators, cephalopods have complex sensory organs, most notably eyes that are comparable to those of vertebrates in their ability to resolve images. The cephalopod head is closely associ-

ated with a large, branched foot that bears tentacles and a siphon. The large, muscular mantle is a solid external supporting structure. The gills hang within the mantle cavity.

Cephalopods appeared about 600 million years ago, near the beginning of the Cambrian period, and by the Ordovician period a wide variety of types were present. They were the first large, shelled animals able to move vertically in the ocean. The earliest cephalopod shells were divided by partitions penetrated by tubes through which liquids could be moved. Nautiloids (genus *Nautilus*) are the only cephalopods with external chambered shells that survive today (Figure 31.26f). Increases in size and reductions in external hard parts characterize the subsequent evolution of many lineages.

ro



Chapter Summary

Descendants of a Common Ancestor

- ▶ All members of the kingdom Animalia are believed to have a common flagellated protist ancestor.
- ▶ The specialization of cells by function made possible the complex, multicellular body plan of animals.

The Animal Way of Life

- ▶ Animals obtain their food—complex organic molecules— by active expenditure of energy.

Clues to Evolutionary Relationships among Animals

- ▶ Morphological, developmental, and molecular data support similar animal phylogenies.
- ▶ The body cavity of an animal is strongly correlated with its ability to move. On the basis of their body cavities, animals are classified as acoelomates, pseudocoelomates, or coelomates. Review Figure 31.1
- ▶ The two major animal lineages—protostomes and deuterostomes—are believed to have separated early in animal evolution; they differ in several components of their early embryological development. Review Figure 31.2

Body Plans Are Basic Structural Designs

- ▶ Most animals have either radial or bilateral symmetry. Radially symmetrical animals move slowly or not at all. Bilateral symmetry is strongly correlated with more rapid movement and the development of sensory organs at the anterior end of the animal. Review Figure 31.3

Sponges: Loosely Organized Animals

- ▶ Sponges (phylum Porifera) are simple animals that lack cell layers and body symmetry, but have several different cell types.
- ▶ Sponges feed via choanocytes, feeding cells that draw water through the sponge body and filter out small organisms and nutrient particles. Review Figure 31.4

Cnidarians: Cell Layers and Blind Guts

- ▶ Cnidarians (phylum Cnidaria) are radially symmetrical and have only two cell layers, but with their nematocyst-studded tentacles they can capture prey larger and more complex than themselves. Review Figure 31.7

ANIMAL ORIGINS AND LOPHOTROCHOZOANS 563

- ▶ Most cnidarian life cycles have a sessile polyp and a free-swimming, sexual medusa stage, but some species lack one of the stages. Review Figures 31.8, 31.9, 31.10

Ctenophores: Complete Guts and Tentacles

- ▶ Ctenophores (phylum Ctenophora), descendants of the first split in the lineage of bilaterally symmetrical animals, are marine carnivores that have simple life cycles. Review Figure 31.12

The Evolution of Bilaterally Symmetrical Animals

- ▶ The common ancestors of bilateral animals, called urbilaterians, were probably simple, bilaterally symmetrical animals composed of flattened masses of cells.

Protostomes and Deuterostomes: An Early Lineage Split

► Protostomes and deuterostomes are monophyletic lineages that have been evolving separately since the Cambrian period. Their members are structurally more complex than cnidarians and ctenophores. Protostomes have a ventral nervous system, paired nerve cords, and larvae with compound cilia. Deuterostomes have a dorsal nervous system and larvae with single cilia.

► Protostomes split into two major clades—lophotrochozoans and ecdysozoans. Review Figure 31.14

Simple Lophotrochozoans

► Flatworms (phylum Platyhelminthes) have no body cavity, lack organs for oxygen transport, have only one entrance to the gut, and move by beating their cilia. Many species are parasitic. Review Figures 31.15, 31.16

► Although no larger than many ciliated protists, rotifers (phylum Rotifera) have highly developed internal organs. Review Figure 31.17

Lophophorates: An Ancient Body Plan

► The lophotrochozoan lineage split into two branches whose descendants became the lophophorates and the spiralian.

► The lophophore dominates the anatomy of many lophophorate animals. Review Figure 31.18

► Bryozoans are colonial lophophorates that can move their lophophores. Review Figure 31.20

► Brachiopods, which superficially resemble bivalve mollusks, were much more abundant in the past than they are today.

Spiralians: Wormlike Body Plans

► The spiralian lineage gave rise to many phyla, most of whose members have wormlike body forms.

► Ribbon worms (phylum Nemertea) have a complete digestive tract and capture prey with an eversible proboscis. Review Figure 31.22

Segmented Bodies: Improved Locomotion

► Annelids (phylum Annelida) are a diverse group of segmented worms that live in marine, freshwater, and terrestrial environments. Review Figure 31.23

► Mollusks (phylum Mollusca) evolved from segmented ancestors but subsequently became unsegmented. The molluscan body plan has three basic components: foot, mantle, and visceral mass. Review Figure 31.25

► The molluscan body plan has been modified to yield a diverse array of animals that superficially appear very different from one another.

For Discussion

1. Differentiate among the members of each of the following sets of related terms:

a. radial symmetry/bilateral symmetry

b. protostome/deuterostome

c. indeterminate cleavage/determinate cleavage

d. spiral cleavage/radial cleavage

e. coelomate/pseudocoelomate/acoelomate

2. For each of the types of organisms listed below, give a single trait that may be used to distinguish them from the organisms in parentheses:

a. cnidarians (sponges)

b. gastropods (all other mollusks)

c. polychaetes (other annelids)

3. In this chapter we listed some of the traits shared by all animals that convince most biologists that all animals are descendants of a single common ancestral lineage. In your opinion, which of these traits provides the most compelling evidence that animals are monophyletic?

4. Describe some features that allow animals to capture prey that are larger and more complex than they themselves are.

5. Animals in many phyla have wormlike, or vermiform, shapes. Why has this body form met the needs of species in so many different lineages of animals? In what types of environments does the worm shape function well? Why?

6. Having a complete digestive tract in which materials enter at an anterior mouth and move in one direction until they exit from a posterior opening would appear to be a very efficient way to digest food and rid the body of the indigestible residues. Nonetheless, several successful phyla of animals with a blind gut must void their digestive wastes via the same opening through which food entered. Why has this type of digestive system persisted? What limitations does it impose on the types of food animals can eat and the way in which the food is treated?

32

Ecdysozoans: The Molting Animals



A FIRM, NONLIVING COVERING THAT IS DIFFICULT to penetrate—an exoskeleton —provides an animal with both protection and support. Its very attributes, however, pose a huge problem: An exoskeleton cannot grow as the animal body inside it grows. Ancestors of today's ecdysozoan animals evolved a solution. They shed, or molt, the outgrown exoskeleton and expand and harden a new, larger one.

The new exoskeleton is already in place, growing underneath the old one. Directly after molting, the animal is very vulnerable. With its soft, new armor it can move only very slowly for a while. Despite this constraint, the lineages of Ecdysozoa—the molting animals—have more species than all other animal lineages combined.

An increasingly rich array of molecular and genetic evidence, including a common set of homeobox genes, suggests that molting may have evolved only once during animal evolution. The exoskeletons of ecdysozoan animals range from thin and flexible to thick, hard, and rigid.

The presence of an exoskeleton presented new problems and opportunities in other areas of the body plan besides growth. Unlike the lophotrochozoans, ecdysozoans cannot use cilia for locomotion; new forms of locomotion evolved in these lineages. And because hard exoskeletons impede the passage of oxygen into the animal, new mechanisms for respiration evolved.

In this chapter we will review the characteristics of animals in the various ecdysozoan phyla and show how developing an exoskeleton has influenced the evolution of these animals. The phylogeny we follow here is presented in Figure 32.1. The latter half of the chapter details the characteristics of several ecdysozoan lineages that have traditionally been classified in the phylum Arthropoda—the arthropods.

Collectively, arthropods (which include the terrestrial insects and the marine crustaceans) are the dominant animals on Earth, both in number of species (some 1.5 million) and number of individuals (estimated at some 10¹⁸ individuals, or a billion billion). The highly successful arthropod body plan is based on three elements: the rigid exoskeleton that marks them as ecdysozoans; segmenta-

Molting the Exoskeleton

This green darner dragonfly (genus *Anax*) has just emerged from its larval exoskeleton and is pumping fluids into its expanding wings. At this stage the insect can move only very slowly.

tion; and jointed appendages, which immensely enhance their powers of locomotion, and which we will encounter again in Chapter 33 when we cover another major lineage, the vertebrates.

We close the chapter with an overview of evolutionary themes found in the evolution of the protostomate phyla, including both the lophotrochozoan and ecdysozoan lineages.

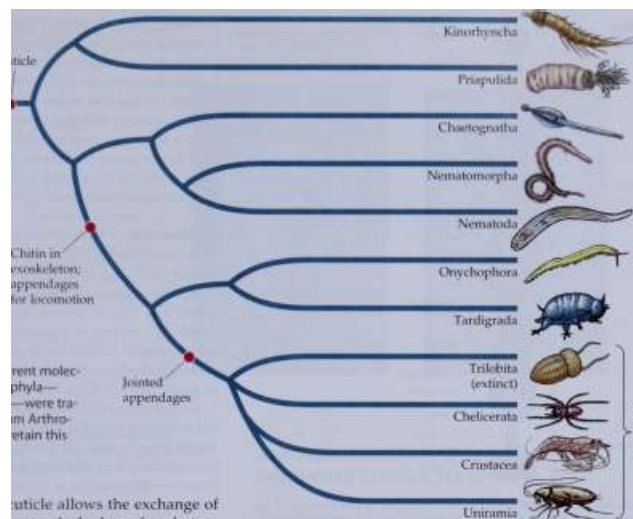
Cuticles: Flexible, Unsegmented Exoskeletons

Some ecdysozoans have wormlike bodies covered by exoskeletons that are relatively thin and flexible. These exoskeletons, called cuticles, protect the animal, but do not provide support for the bodies. The action of circular and longitudinal muscles on fluid in the body cavity provides a hydrostatic skeleton for many of these animals, which can



Cuticle

Ecdysozoan ancestor



Jointed appendages

Chitin in exoskeleton;

appendages for locomotion

32.1 A Probable Phylogeny of Ecdysozoans

This approach is in line with current molecular evidence. The bottom four phyla—those with jointed appendages—were traditionally classified as the phylum Arthropoda, and many classifications retain this distinction.

move only slowly. A thin cuticle allows the exchange of gases, minerals, and water across the body surface, but restricts the animal to moist habitats.

Some marine phyla have few species

Several phyla of marine wormlike animals (that is, they are long and slender, without appendages) branched off early within the ecdysozoan lineage. These phyla contain only a few species. They have relatively thin cuticles that are molted periodically as they grow to full size. Their bodies are supported primarily by their hydrostatic skeletons, not by the cuticle.

priapulids and kinorhynchs. The 16 species of priapulids (phylum Priapulida) are cylindrical, unsegmented, wormlike animals that range in size from half a millimeter to 20 centimeters in length. They burrow in fine marine sediments.

About 150 species of kinorhynchs (phylum Kinorhyncha) have been described. They are all less than 1 millimeter in length and live in marine sands or muds. Their bodies are divided into 13 segments by a series of cuticular plates that are periodically molted during growth (Figure 32.2). Kinorhynchs feed by ingesting the substratum and digesting the organic material found within it, which may include living algae as well as dead matter.

arrow worms. Arrow worms (phylum Chaetognatha)

have three-part, streamlined bodies. Their body plan is based on a coelom that is divided into head, trunk, and tail compartments. Most of them swim in the open sea, but a few live on the seafloor. Their abundance as fossils indicates that they were already common more than 500 million years ago. The 100 or so living species of arrow worms are so small—less than 12 centimeters long—that their gas exchange and excretion requirements can be met by diffusion through the body surface. Arrow worms lack a circulatory system. Wastes and nutrients are moved around the body in the coelomic fluid, which is propelled by cilia that line the coelom. Arrow



Nematomorpha

Nematoda

Onychophora

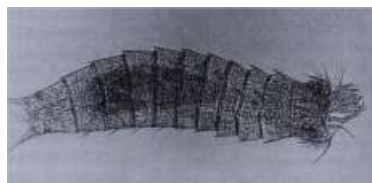
Tardigrada

Trilobita

Chelicerata

Crustacea

Uniramia



32.2 A Kinorhynch

Kinorhynchs are tiny (less than a millimeter long) marine worms. Their segmented bodies are covered with plates of cuticle that are periodically molted.

566 CHAPTER THIRTY-TWO

I load r

compartment \

Trunk compartment' 4

Tail compartment

Grasping spines

Head

Trunk

Anterior lateral fin

Ovary

Posterior lateral fin

Anus

- Trunk-tail partition

Testis

Seminal vesicle

- Tail fin

32.3 An Arrow Worm

Arrow worms have a three-part body plan. The fins and grasping spines are adaptations for a predatory life.

worms are stabilized in the water by means of one or two pairs of lateral fins and a "tail" fin (Figure 32.3). There is no distinct

larval stage; miniature adults hatch directly from eggs that are released into the water.

Arrow worms are major predators of small organisms in the open oceans. Their prey range from small protists to young fish as large as an arrow worm. An arrow worm typically lies motionless in the water until movement of the water signals the approach of prey. The arrow worm then darts forward and grasps the prey with the stiff spines adjacent to its mouth.

Kinorhyncha

Priapulida

Chaetognatha

Nematomorpha

Tough cuticles evolved in some unsegmented worms ^^

Tough external cuticles evolved in some members of an ecdysozoan lineage whose descendants have colonized freshwater

and terrestrial environments

Two extant phyla represent this lineage.

Chelicerata HORSEHAIR WORMS. About 230 species

of horsehair worms (phylum Nematomorpha) have been described. As their name

implies, they are extremely thin and range in length from a

few millimeters to up to a meter (Figure 32.4). Most adult horsehair worms live in fresh water among litter and algal mats

near the edges of streams and ponds. The larvae of horsehair worms are all internal parasites of terrestrial and aquatic

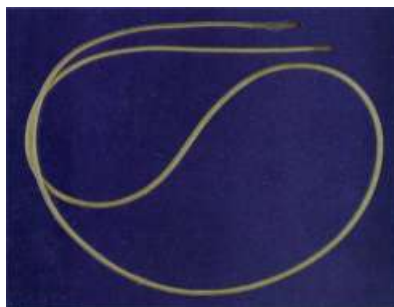
insects and crabs. The much reduced gut has no mouth opening and is probably nonfunctional. Horsehair worms may feed

only as larvae, absorbing nutrients from their hosts across their body wall, but many continue to grow after they have left their hosts, suggesting that adults may also absorb nutrients from their environment.

Roundworms. Roundworms (phylum Nematoda) have a thick, multilayered cuticle secreted by the underlying epidermis that gives their body its shape (Figure 32.5). As a roundworm grows, it sheds and re-secretes its cuticle four times. The largest known roundworm, which reaches a length of 9 meters, is a parasite in the placentas of female sperm whales. About 20,000 species of roundworms have been described, but the actual number of living species may be more than a million.

Roundworms exchange oxygen and nutrients with their environment through both the cuticle and the intestine, which is only one cell layer thick. Materials are moved through the gut by rhythmic contraction of a highly muscular organ, the pharynx, at the worm's anterior end. Roundworms move by contracting their longitudinal muscles.

Roundworms are one of the most abundant and universally distributed of all animal groups. Countless roundworms live as scavengers in the upper layers of the soil, on the bottoms of lakes and streams, and as parasites in the bodies of most kinds of plants and animals. The flesh of a single rotting apple found on the ground in an orchard contained 90,000 roundworms, and 1 square meter of mud off the coast of the Netherlands yielded 4,420,000 individuals. The topsoil of rich farmland has up to 3 billion nematodes per acre.

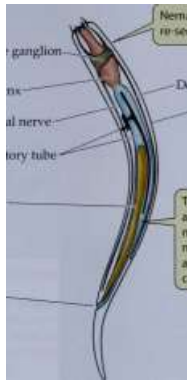


32.4 A Horsehair Worm

How these worms got their name is evident from this photograph.

Anus

(a)



Nematodes grow, shed, and re-secrete their cuticle four times.

Nerve ganglion

Pharynx Ventral nerve Excretory tube

Testis

A cyst of *Trichinella spiralis* infects its host's muscle tissue.

Dorsal nerve Cuticle

The large gut (blue) and testis (orange) fill most of the body of a male *Trichinella spiralis*, a nematode that causes trichinosis.



This free-living roundworm moves through marine sediments.

(b)

32.5 Nematodes

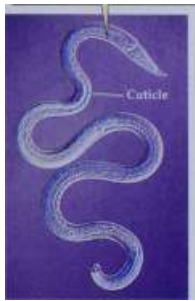
{a,b) *Trichinella* is an example of a parasitic roundworm that infects mammals, including humans, (c) Free-living roundworms have a body plan similar to the adult parasite's.

The diets of roundworms are as varied as their habitats. Many are predators, preying on protists and other small animals (including other roundworms). Many roundworms live parasitically within their hosts. The roundworms that are parasites of humans (causing diseases such as trichinosis, filariasis, and elephantiasis), domestic animals, and economically important plants have been studied intensively in an effort to find ways of controlling them. One soil-inhabiting nematode species, *Caenorhabditis elegans*, has been intensely studied in the laboratory by geneticists and developmental biologists.

The structure of parasitic roundworms is similar to that of free-living species, but the life cycles of many parasitic species have special stages that facilitate their transfer among hosts. *Trichinella spiralis*, the species that causes the human disease trichinosis, has a relatively simple life cycle. A person may become infected by eating the flesh of an animal (usually a pig) containing larvae of *Trichinella* encysted in its muscles.

The larvae are activated in the mammalian digestive tract, leave their cysts, and attach to the person's intestinal wall, where they feed. Later they bore through the intestinal wall and are carried in the bloodstream to the muscles, where they form cysts (Figure 32.5b). If present in great numbers, these cysts can cause severe pain or even death.

Trichinella can infect a number of mammal species; there is no special stage in the life cycle that lives in a particular alternate host. Other roundworm life cycles are more complex, involving one or more alternate hosts.



(c)

Arthropods and Their Relatives: Segmented External Skeletons

In Precambrian times, the body coverings of some wormlike ecdysozoan lineages became thickened by the incorporation of layers of protein and a strong, flexible, waterproof polysaccharide called chitin. After this change, which initially probably had a protective function, the rigid body covering acquired both support and locomotory functions.

A rigid body covering precludes wormlike movement. To move, these animals require appendages that can be manipulated by muscles. Such appendages evolved several times in late Precambrian times, leading to the phyla collectively called arthropods. The divisions among arthropod lineages are so ancient that we divide them into a number of phyla. However, as indicated at the opening of this chapter, many zoologists treat these groups as members of a single phylum, Arthropoda.

The bodies of arthropods are divided into segments. Their muscles attach to the inside of the skeleton, and each segment has muscles that operate that particular segment and the appendages attached to it (Figure 32.6). The appendages of most present-day arthropods have joints, although those of some lineages do not. Arthropod appendages serve many functions, including walking and swimming, food capture and manipulation, copulation, and sensory perception.

The sturdy exoskeleton had a profound influence on arthropod evolution. Encasement within armor provides support for walking on dry land, and, with special waterproofing, it keeps the animal from dehydrating in dry air. Aquatic arthropods were, in short, excellent candidates to invade the terrestrial environment, and as we will see, they did so several times.

568 CHAPTER THIRTY-TWO

Hemocoel Heart

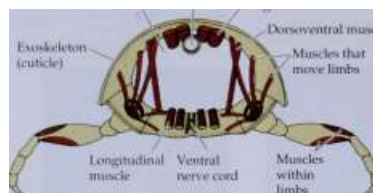
Exoskeleton

(cuticle)

Longitudinal muscle

Dorsoventral muscle

Muscles that move limbs



32.6 Arthropods Have Rigid, Segmented Exoskeletons

This cross section through a segment of a generalized arthropod shows the typical structure of an arthropod body, which is characterized by a rigid exoskeleton and jointed appendages.

Related lineages had unjointed legs

Although they were once thought to be closely related to

Kinorhyncha

Priapulida

Chaetognatha

Nematomorpha

Nematoda



segmented annelid worms, the molecular evidence links the 80 species of onychophorans (phylum Onychophora) to the arthropod lineages. Onychophorans have soft bodies that are covered by a thin, flexible cuticle that contains chitin. Onychophorans use their body cavities as hydrostatic skeletons. Their soft, fleshy, unjointed, claw-bearing legs are formed by outgrowths of the body (Figure 32.7a). They are probably similar in appearance to ancestral arthropods.

Like the onychophorans, water bears (phylum Tardigrada) have fleshy, unjointed legs and use their fluid-filled body cavities as hydrostatic skeletons (Figure 32.7b). Unlike onychophorans, water bears are all extremely small (0.1-0.5 mm in length), and they lack circulatory systems and gas exchange organs. The 600 extant species of water bears live in marine sands and on temporary water films on plants. When these films dry out, the water bears also lose water and shrink to small, barrel-shaped objects that can survive for at least a decade in a dehydrated resting state. They may occur at densities as high as 2,000,000 per square meter of moss.

Jointed legs appeared in the trilobites

Once the dominant line of arthropods, the trilobites (phylum Trilobita) flourished in Cambrian and Ordovician seas but were extinct by the close of the Paleozoic era. Trilobites were heavily armored, and their body segmentation and appendages followed a relatively simple, repetitive plan. But their appendages were jointed, giving them led flexibility, and the beginnings of specialization— ig different appendages for different functions—can be discerned.



(a) *Peripatodes novaezealandiae*



(b) *Echiniscus springer*

50 urn

32.7 Arthropod Relatives with Unjointed Appendages

(a) Onychophorans have unjointed legs and use the body cavity as a hydrostatic skeleton, (b) The appendages and general anatomy of a water bear (phylum Tardigrada) superficially resemble those of onychophorans.

Why trilobites declined in abundance and eventually became extinct is unknown. However, because their heavy external skeletons provided ideal material for fossilization, they left behind a vivid record of their presence (Figure

32.8).



Odontochile rugosa

32.8 A Trilobite

The relatively simple, repetitive segments of the now-extinct trilobites are illustrated here by a fossil trilobite from the

shallow seas of the Devonian period.



(a) *Decapoda* sp.

32.9 Minor Chelicerate Phyla

(a) Although they are not true spiders, it is easy to see why sea spiders were given their common name, (b) This spawning aggregation of horseshoe crabs was photographed on the New Jersey coast.

(b) *Limulus polyphemus*

Chelicerates Invaded the Land

The bodies of all chelicerates (phylum Chelicerata) are divided into two major regions. The anterior region bears two pairs of appendages, modified to form mouthparts, and four pairs of walking legs. The 63,000 described species are usually placed in three classes: Pycnogonida, Arachnida, and Merostomata. Only the class Arachnida contains many species.

The pycnogonids (class Pycnogonida), or sea spiders, are a small group of marine species that are seldom seen except by marine biologists (Figure 32.9a). The class Merostomata contains a single order, the Xiphosura, or horseshoe crabs. These marine animals, which have changed very little during their long fossil history, have a large horseshoe-shaped covering over most of the body. They are common in shallow waters along the eastern coasts of North America and Southeast Asia, where they scavenge and prey on bottom-dwelling invertebrates. Periodically they crawl into the intertidal zone to mate and lay eggs (Figure 32.9b).

Arachnids (class Arachnida) are abundant in terrestrial environments. Most arachnids have a simple life cycle in which miniature adults hatch from eggs and begin independent lives almost immediately. Some arachnids retain their eggs during development and give birth to live young. The most species-rich and abundant arachnids are the scorpions, harvestmen, spiders, mites, and ticks (Figure 32.10).

Spiders are important terrestrial predators. Some have excellent vision that enables them to chase and seize their prey. Others spin elaborate webs made of protein threads to



Kinorhyncha Priapulida Chaetognatha Nematomorpha Nematoda Onychophora Tardigrada Trilobita

Chelicerata

Crustacea Uniramia

snare prey. The webs of different groups of spiders are strikingly varied and enable spiders to position their snares in many different environments. Spiders also use protein threads to construct safety lines during climbing and as homes, mating structures, protection for developing young, and means of dispersal. The threads are produced by modified abdominal appendages that are connected to internal glands that secrete the proteins of which the threads are constructed.

Crustaceans: Diverse and Abundant

Crustaceans (phylum Crustacea) are the dominant marine arthropods. The most familiar crustaceans are decapods (shrimps, lobsters, crayfishes, and crabs; Figure 32.11f); isopods (sow bugs; Figure 32.11b); and amphipods (sand fleas; see Figure 1.11). Also included among the crustaceans are a wide variety of other small species, many of which superficially resemble shrimps (Figure 32.11c). The individuals of one group alone, the copepods (class Cope-poda), are so numerous that they may be the most abundant of all animals.

Barnacles (class Cirripedia) are unusual crustaceans that are sessile as adults (Figure 32.1d). With their calcareous shells, they superficially resemble mollusks, but, as the zoologist Louis Agassiz remarked more than a century ago, a barnacle is "nothing more than a little shrimp-like animal, standing on its head in a limestone house and kicking food into its mouth."

Kinorhyncha

Priapulida

Chaetognatha

Nematomorpha

Nematoda

Onychophora

Tardigrada

Trilobita



Uniramia



32.10 Diversity among the Arachnids

(a) Scorpions are nocturnal predators, (b) Many spiders use webs to snare and envelop their prey. This ogre-faced spider uses its specialized web like a net. (c) Harvestmen, often called daddy longlegs, are scavengers, (d) Ticks are bloodsucking, external parasites on vertebrates. This wood tick is piercing the skin of its human host.

(b) *Deinopis* sp.

(d) *Ixodes ricinus*

(a) *Orconectes palmeri*



(b) *Armadillidium vulgare*

32.11 Diversity among the Crustaceans

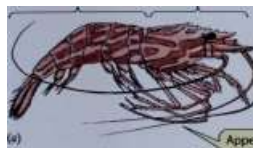
(a) This crayfish is a decapod crustacean, (b) This sow bug is a common isopod found in grasslands, (c) A typical planktonic copepod from the deep ocean, (d) The appendages of these gooseneck barnacles protrude from their shells to capture prey.

(c) *Megacalanus princeps*



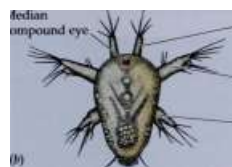
Abdomen

Carapace covering head and thorax



Appendages are specialized for chewing, sensing, walking, and swimming.

Median compound eye



Antennule

Antenna

Mandible

32.12 Crustacean Structure

(a) The bodies of most crustaceans are divided into three regions, each segment of which bears appendages, (b) A nauplius larva has one simple eye and three pairs of appendages.



Myriapods have many legs

Centipedes, millipedes, and the two other groups of animals in the subphylum Myriapoda have two body regions: a head and a trunk. Centipedes and millipedes have a well-formed head and a long, flexible, segmented trunk that bears many pairs of legs (Figure 32.13). Centipedes prey on insects and other small animals. Millipedes scavenge and eat plants. More than 3,000 species of centipedes and 10,000 species of millipedes have been described; many more species probably remain unknown. Although most myriapods are less than a few centimeters long, some tropical species are ten times that size.

Insects are the dominant uniramians

The 1.5 million species of insects (subphylum Insecta) that have been described are believed to be only a small fraction of the total number living on Earth today. Insects are found in nearly all terrestrial and freshwater habitats, and they utilize as food nearly all species of plants and many species of animals. Some are internal parasites of plants and animals; others suck their host's blood or consume its surface

Kinorhyncha Priapulida Chaetognatha ematomorpha toda Onychophora Tardigrada TriJobita Chelicerata Crustacea



Most of the 40,000 described species of crustaceans have a body that is divided into three regions: head, thorax, and abdomen. The segments of the head are fused together, and the head bears five pairs of appendages. Each of the multiple thoracic and abdominal segments usually bears one pair of appendages. In many species, a fold of the exoskeleton, the carapace, extends dorsally and laterally back from the head to cover and protect some of the other segments (Figure 32.12a).

The fertilized eggs of most crustacean species are attached to the outside of the female's body, where they remain during their early development. At hatching, the young of some species are released as larvae; those of other species are released as juveniles that are similar in form to the adults. Still other species release fertilized eggs into the water or attach them to an object in the environment. The typical crustacean larva, called a nauplius, has three pairs of appendages and one simple eye (Figure 32.12b). In many crustaceans, the nauplius larva develops within the egg before it hatches.

Uniramians are Primarily Terrestrial

The body of a uniramian (phylum Uniramia) is divided into either two or three regions (in myriapods and insects, respectively). The anterior regions have few segments, but the posterior region—the abdomen—has many segments. Uniramians are primarily terrestrial animals; most have elaborate systems of channels that bring oxygen to the cells of their internal organs.



(a) *Scolopendra heros*



(b) *Harapaphe haydeniana*

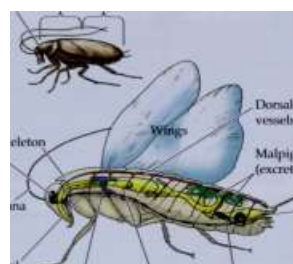
32.13 Myriapods

(a) Centipedes have powerful jaws for capturing active prey.

(fc>) Millipedes, which are scavengers and plant eaters, have smaller jaws and legs.

572 CHAPTER THIRTY-TWO

Head Thorax Abdomen



Dorsal circulatory vessels

Exoskeleton Brain

Antenna

Ventral

nerve cord Trachea

Malpighian tubules (excretory organs)

Anus

Gut

Testis

32.14 Structure of an Insect

The body plan of an insect differs in many details from that of other arthropods, but the basic theme of a segmented body with modified jointed appendages is shared with most arthropod lineages.

body tissues. Insects transmit many viral, bacterial, and protist diseases among plants and animals. Very few insect species are oceanic. In freshwater environments, on the other hand, they are sometimes the dominant animals, burrowing through the muddy substrate, extracting suspended prey from the water, or actively pursuing other animals.

Insects, like the crustaceans, have three basic body parts: head, thorax, and abdomen. They have a single pair of antennae on the head, and three pairs of legs attached to the thorax (Figure 32.14). An insect exchanges gases by means of air sacs and tubular channels called tracheae (singular trachea) that extend from external openings inward to tissues throughout the body. The adults of most flying insects have two pairs of stiff, membranous wings attached to the thorax. The exceptions are flies, which have only one pair of wings, and beetles, in which the forewings form heavy, hardened wing covers.

Wingless insects (class Apterygota) include firebrats and collembolans (Figure 32.15a). Of the modern insects, they are probably the most similar in form to insect ancestors. Apterygote insects have a simple life cycle, hatching from their eggs looking like small adults.

Development in the winged insects (class Pterygota) is more complex. The hatchlings are not similar to adults, and they undergo substantial changes at each molt in the process of growing larger. The immature stages of insects between molts are called instars. A substantial change that occurs between one developmental stage and another is called metamorphosis. When the change from one instar to the next is gradual, an insect is said to undergo incomplete metamorphosis.

In some insect genera, the larvae and adult forms can appear to be completely different animals. The most familiar example of such complete metamorphosis occurs in members of the order Lepidoptera, when the larval caterpillar trans-

forms itself into the adult butterfly (see Figure 1.6). During complete metamorphosis, the wormlike larva transforms itself during a specialized phase, called the pupa, in which many larval tissues are broken down and the adult form develops.

Entomologists divide the winged insects into about 28 different orders. We can make sense out of this bewildering variety by recognizing three major lineages:

- ▶ Winged insects that cannot fold their wings back against the body.
- ▶ Winged insects that can fold their wings and that undergo incomplete metamorphosis.
- ▶ Winged insects that can fold their wings and that undergo complete metamorphosis.

Because they can fold their wings over their backs, flying insects belonging to the second and third lineages are able to tuck their wings out of the way upon landing and crawl into crevices and other tight places.

The only surviving groups of the first lineage are the orders Odonata (dragonflies and damselflies; Figure 32.15b) and Ephemeroptera (mayflies). All members of these two orders have aquatic larvae that metamorphose into flying adults after they crawl out of the water. Although many of these insects are excellent flyers, they require a great deal of open space in which to maneuver. Dragonflies and damselflies are active predators as adults, but adult mayflies lack functional digestive tracts and do not eat, living only long enough to mate and lay eggs.

The second lineage includes the orders Orthoptera (grasshoppers, crickets, roaches, mantids, and walking sticks; Figure 32.15c), Isoptera (termites), Plecoptera (stone flies), Dermaptera (earwigs), Thysanoptera (thrips), Hemiptera (true bugs; Figure 32.15d), and Homoptera (aphids, cicadas, and leafhoppers). Hatchlings are sufficiently similar in form to adults to be recognizable. They acquire adult organ systems, such as wings and compound eyes, gradually through several juvenile instars.

Insects belonging to the third lineage have different life stages specialized for living in different environments and using different food sources. In many species the larvae are adapted for feeding and growing, and the adults are specialized for reproduction and dispersal. The adults of some species do not feed at all, living only long enough to mate, disperse, and lay eggs. In many species whose adults do feed, adults and larvae use different food resources. About 85 percent of all species of winged insects belong to this lineage. Familiar examples are the orders Neuroptera (lacewings

32.15 Diversity among the Insects \

(a) This silverfish is a typical member of the apterygote order Thysanura. (b) Unlike most insects, this adult dragonfly (order Odonata) cannot fold its wings over its back. Representatives of some of the largest insect orders are (c) a broad-winged katydid (Orthoptera), (d) harlequin bugs (Hemiptera), (e) a predaceous diving beetle (order Coleoptera), (f) a Great Mormon butterfly (Lepidoptera), (g) a hoverfly (Diptera), and (h) a honeybee (Hymenoptera).

ECDYSOZOANS:THE MOLTING ANIMALS 573



(d) Murgantia histrionica

(h) Apis mellifera

574 CHAPTER THIRTY-TWO

"All have bilateral symmetry.

and their relatives), Coleoptera (beetles; Figure 32.15e), Tri-choptera (caddisflies), Lepidoptera (butterflies and moths; Figure 32.15f), Diptera (flies; Figure 32.15g), and Hymenoptera (sawflies, bees, wasps, and ants; Figure 32.15h).

There are also several orders of pterygote insects, including the Phthiraptera (lice) and Siphonaptera (fleas) that are parasitic. Although descended from flying ancestors, these insects have lost the ability to fly.

Why have the insects undergone such incredible evolutionary diversification? Insects may have originated from a centipede-like ancestor as far back as the Devonian period. The terrestrial environments penetrated by the arthropods were like a new planet, an ecological world with more complexity than the seas they came from, but one containing relatively few species other than the insects. The evolution of the ability to fly allowed the insects to escape from potential predators and to traverse boundaries that might otherwise have been insurmountable—both very highly adaptive features. The numbers and diversity of insect species attest to the supreme success of this highly visible and dominant animal group.

Themes in Protostome Evolution

Most of protostome evolution took place in the oceans. As we have seen, early animals used fluids within their body cavities as the basis for support and movement. Subdivisions of the body cavity allowed better control of movement and permitted different parts of the body to be moved independently of one another. Thus some protostome lineages gradually evolved the ability to change their shape in complex

ways and to move with greater speed on and through sediments or in the water.

During much of animal evolution, the only food available in the water consisted of dissolved organic matter and very small organisms. Consequently, many different lineages of animals evolved feeding structures designed to extract small prey from water, as well as structures for moving water through or over their prey-collecting devices. Animals that feed in this manner are abundant and widespread in marine waters today.

Because water flows readily, bringing food with it, sessile lifestyles evolved repeatedly during lophotrochozoan and ecdysozoan evolution. Most protostome phyla today have at least some sessile members. Sessile lifestyles have both advantages and disadvantages. A sessile animal gains access to local resources, but forfeits access to more distant resources. Sessile animals cannot come together to mate; instead, they must rely on the fertilization of gametes that they have ejected into the water. Some species eject both eggs and sperm into the water; others retain their eggs within their bodies and extrude only their sperm, which are carried by the water to other individuals. Species whose adults are sessile often have motile larvae, many of which have complicated mechanisms for locating suitable sites on which to settle. Many colonial sessile protostomes are able to grow in the direction of better resources or into sites offering better protection.

A frequent consequence of a sessile existence is competition for space. Such competition is intense among plants in most terrestrial environments. In the sea, especially in shallow waters, animals also compete directly for space. They

ECDYSOZOANS:THE MOLTING ANIMALS 575

have evolved mechanisms for overgrowing one another and for engaging in toxic warfare where they come into contact.

Individual members of sessile colonies, if they are directly connected, can share resources. The ability to share resources enables some individuals to specialize for particular functions, such as reproduction, defense, or feeding. The nonfeeding individuals derive their nutrition from their feeding associates.

Predation may have been the major selective pressure behind the development of external body coverings. Such coverings evolved independently in many lophotrochozoan and ecdysozoan lineages. In addition to providing protection, they became key elements in the development of new systems of locomotion. Locomotory abilities permitted prey to escape more readily from predators, but also allowed predators to pursue their prey more effectively. Thus, the evolution of animals has been, and continues to be, a complex arms race among predators and prey.

Although we have concentrated on the evolution of greater complexity in animal lineages, many lineages that remained simple have been very successful. Cnidarians are common in the oceans; roundworms abound in most aquatic and terrestrial environments. Parasites lost complex body plans but evolved complex life cycles.

The characteristics of the major existing phyla of proto-stomate animals are summarized in Table 32.1. All the phyla had evolved by the Cambrian period, but extinction and diversification within these lineages continue.

Many of the evolutionary trends demonstrated by proto-stomes also dominated the evolution of deuterostomes, the lineage that includes the chordates, the group to which humans belong. Hard external body coverings evolved and were later abandoned by many lineages. We will consider the evolution of the deuterostomes in the next chapter.

\ra



Chapter Summary

- ▶ A major innovation during animal evolution was the development of a sturdy, nonliving external cover—an exoskeleton. An animal with an exoskeleton grows by periodically molting its exoskeleton and replacing it with a larger one.
- ▶ The presence of an exoskeleton opened avenues for the evolution of new body plans in the ecdysozoan lineage. Review Figure 32.1

Animals with Flexible Exoskeletons

- ▶ Tough cuticles are found in members of two phyla that live in freshwater, marine, and terrestrial environments.
- ▶ Roundworms (phylum Nematoda) are one of the most abundant and universally distributed of all animal groups. Many are parasites. Review Figure 32.5

Arthropods and Their Relatives: Segmented External Skeletons

- ▶ The body coverings of one ecdysozoan lineage, the arthropods, became thickened and made rigid by the incorporation of layers of protein and the polysaccharide chitin.
- ▶ Animals with rigid exoskeletons cannot move in a wormlike fashion. To move, they have appendages that can be

manipulated by muscles. Review Figure 32.6

- ▶ Onychophorans have soft, fleshy, unjointed legs. They are probably similar to ancestral arthropods.
- ▶ The tiny and abundant water bears (phylum Tardigrada) also have unjointed legs.
- ▶ Jointed legs with specialized functions appeared among the trilobites (phylum Trilobita). Trilobites flourished in Cambrian and Ordovician seas, but became extinct by the close of the Paleozoic era.

Chelicerates: Invasion of the Land

- ▶ The bodies of all chelicerates (phylum Chelicerata) are divided into two major regions, the anterior of which bears four pairs of jointed legs.
- ▶ Arachnids—scorpions, harvestmen, spiders, mites, and ticks—are abundant in terrestrial environments.

Crustaceans: Diverse and Abundant

- ▶ Most of the 40,000 described species of crustaceans (phylum Crustacea) have a body that is divided into three regions: head, thorax, and abdomen. Review Figure 32.12
- ▶ The most familiar crustaceans are shrimps, lobsters, crayfishes, crabs, sow bugs, and sand fleas.

Uniramians are Primarily Terrestrial

- ▶ The body of a uniramian (phylum Uniramia) is divided into two or three regions; the posterior region has paired legs.
- ▶ Myriapods (centipedes and millipedes) have many segments and many pairs of legs.
- ▶ About 1.5 million species of insects (subphylum Insecta) have been described, but that is probably only a small fraction of the total number of species living on Earth today.
- ▶ Insects have three body regions (head, thorax, abdomen), a single pair of antennae on the head, and three pairs of legs attached to the thorax. Review Figure 32.14
- ▶ Wingless insects (class Apterygota) look like little adults when they hatch from their eggs. Hatchlings of many winged insects (class Pterygota) do not resemble adults and undergo substantial changes at each molt.
- ▶ Entomologists divide the winged insects into three major subgroups and about 28 different orders. Members of one subgroup cannot fold their wings back against the body; members of the other two groups can.

Themes in Protostome Evolution

- ▶ Most evolution of protostomes took place in the oceans.
- ▶ Early animals used fluid-filled spaces as hydrostatic skeletons. Subdivision of the body cavity allowed better control of movement and permitted different parts of the body to be moved independently of one another.
- ▶ Predation may have been the major selective pressure for the development of hard, external body coverings.
- ▶ Early in animal evolution, the only food in the water consisted of dissolved organic matter and very small organisms.
- ▶ Flowing water brings food with it, so many animals are sessile.
- ▶ All the phyla of protostomate animals had evolved by the Cambrian period.

576 CHAPTER THIRTY-TWO

For Discussion

1. Segmentation has arisen several times during animal evolution. What advantages does segmentation provide? Given these advantages, why do so many unsegmented animals survive?
2. Many animals extract food from the surrounding medium. What phyla contain animals that extract suspended food from the water column? What structures do these animals use to capture prey?
3. An animal that sheds its external skeleton in order to grow in size is virtually helpless during the time that its new, larger exoskeleton is hardening. Give some examples of how predators take advantage of this vulnerable stage of their prey. Include at least one example of predation by humans.
4. The British biologist J. B. S. Haldane is reputed to have quipped that "God was unusually fond of beetles." Beetles are, indeed, the most species-rich lineage of organisms. What features of beetles have contributed to the generation and survival of so many species? In Part Three we pointed out that major structural novelties have arisen infrequently during the course of evolution. Which of the features of

protostomes do you think are major evolutionary novelties? What criteria do you use to judge whether a feature is a major as opposed to a minor novelty?

A frequent consequence of sessile existence is competition for space. How do plants and animals differ in the ways in which they compete for space?

There are more described and named species of insects than of all other animals lineages combined. However, only a very few species of insects live in marine environments, and those species are restricted to the intertidal zone or the ocean surface. What factors may have contributed to the inability of insects to be successful in the oceans?

33

^/^/ Deuterostome Animals



^ There are about 25,000 species of ray-finned fishes—more species than exist in all other vertebrate groups combined. Ray-finned fishes include almost all fish species with bony skeletons (as opposed to the sharks, whose skeletons are made of cartilage). Most ray-finned fishes have excellent color vision, and they use their brightly colored bodies to advertise their presence, species identity, and sex. Some go through dramatic color changes at different stages of their lives, and some are even able to change colors quickly when they are ready to mate, fight, or flee.

Part of the reason for the richness of ray-finned species may be that fishes are an ancient lineage that has had many millions of years in which to radiate in Earth's oceans and fresh waters. But part of the reason may be genetic. Most vertebrates have only four clusters of homeobox genes, but some ray-finned fishes have seven. The entire genome of these fishes was apparently duplicated about 300 million years ago, providing new opportunities for genetic variability that may have helped drive their explosive evolutionary radiation.

There are fewer major lineages and many fewer species among deuterostomes than among protostomes (Table 33.1), but we have a special interest in deuterostomes because we are members of that lineage. In this chapter, we first discuss some evolutionary themes shared by protostomes and deuterostomes, then describe and discuss the deuterostome phyla Echinodermata, Hemichordata, and Chordata, with special attention to the primate lineage of Chordata that gave rise to our own species.

Deuterostomes and Protostomes: Shared Evolutionary Themes

Deuterostome evolution paralleled protostome evolution in several important ways. Both lineages exploited the abundant food supplies buried in soft marine sediments, attached to rocks, or suspended in water. Because of the ease with which water can be moved, many groups in both lineages developed elaborate structures for moving water and extracting prey from it.

Two Colors, One Fish

The spotted puffer fish, *Arothron meleagris*, changes color during the course of its life cycle. The individuals shown here are in two different color phases of the cycle.

In lineages of both groups, the body became divided into compartments that allowed better control of shape and movement. Some members of both groups evolved mechanisms for controlling their buoyancy in water, using gas-filled internal spaces. Planktonic larval stages evolved in marine members of many protostome and deuterostome phyla; these all fed on tiny planktonic organisms while floating freely in the open water.

The ancestral traits shared by all members of the deuterostome lineage include indeterminate cleavage in the early embryo, a blastopore that becomes the anus, three body layers (they are triploblastic), formation of the mesoderm from an outpocketing of the embryonic gut, and a well-developed coelom (see Chapter 31). No fossils of ancestral deuterostomes that lived before the lineage split into two major lineages (echinoderms and chordates; Figure 33.1) have been found, so we can only deduce what they must have been like from these shared traits.

Both protostomes and deuterostomes colonized the land—the former via beaches, the latter via fresh water— but the consequences of these colonizations were very different. The jointed external skeletons of arthropods, although they provide excellent support and protection in air, cannot support large animals. The internal skeletons developed by deuterostomes are capable of supporting large bodies. The largest terrestrial deuterostomes to ever live were some of the dinosaurs; elephants are the largest living terrestrial animals.

Terrestrial deuterostomes recolonized aquatic environments a number of times. Suspension feeding re-evolved in several of these lineages. The largest living animals, the baleen (toothless) whales, feed on relatively small prey that they extract from the water with large straining structures in their mouths.

Merostomata: Horseshoe crabs Arachnida: Scorpions, harvestmen, spiders,

mites, ticks Crabs, shrimps, lobsters, barnacles, copepods Myriapoda: Millipedes, centipedes Insecta: Insects

Echinodermata: Echinoderms

7,000

DEUTEROSTOMES

Crinoidea: Sea lilies, feather stars Ophiuroidea: Brittle stars Asteroidea: Sea stars Concentricycloidea: Sea daisies Echinoidea: Sea urchins Holothuroidea: Sea cucumbers

Hemichordata: Hemichordates 85

Chordata: Chordates 50,000

Acorn worms

Urochordata: Sea squirts Cephalochordata: Lancelets Agnatha: Lampreys, hagfishes Chondrichthyes: Cartilaginous fishes Osteichthyes: Bony fishes Amphibia: Amphibians Reptilia: Reptiles Aves: Birds Mammalia: Mammals

"Some small phyla are not included.



33.1 A Probable Deuterostomate Phylogeny

There are fewer major lineages and many fewer species of deuterostomes than of protostomes.

Echinoderms: Complex Biradial Symmetry

The ancestors of one deuterostome lineage, the echinoderms (phylum Echinodermata), were probably sluggish animals. They evolved into more aggressive and active forms as a result of two major structural features. One is a system of calcified internal plates covered by thin layers of skin and some muscles. The calcified plates of early echinoderm ancestors became enlarged and thickened until they fused inside the entire body, giving rise to an internal skeleton.

Hemichordata

Urochordata (tunicates)

Cephalochordata

Vertebrata

The other major innovation was the evolution of a water vascular system, a network of calcified hydraulic canals leading to extensions called tube feet. The water vascular system functions in gas exchange, locomotion, and feeding (Figure 33.2a). Seawater enters the water vascular system through a perforated sieve plate. A calcified canal leads from the sieve plate to another canal that rings the esophagus. Other canals radiate from this ring canal extending through the arms (in species that have arms) and connecting with the tube feet. The development of these two structural innovations—calcified internal skeleton and water vascular system—resulted in one of the most striking of evolutionary radiations.

Echinoderms have an extensive fossil record. About 23 classes have been described, of which only 6 survive today. About 7,000 species of echinoderms exist today, but 13,000 species—probably only a small fraction of those that actually lived—have been described from their fossil remains. Nearly all living species have a bilaterally symmetrical, ciliated larva that feeds for some time as a planktonic organism before settling and transforming into a biradially symmetrical adult (Figure 33.2b).

The living echinoderms are divided into two lineages: Pelmatozoa and Eleutherozoa. The two lineages differ in the number of arms they have and the form of their water vascular systems. Pelmatozoa consists only of the crinoids, whereas several groups are included in the Eleutherozoa.

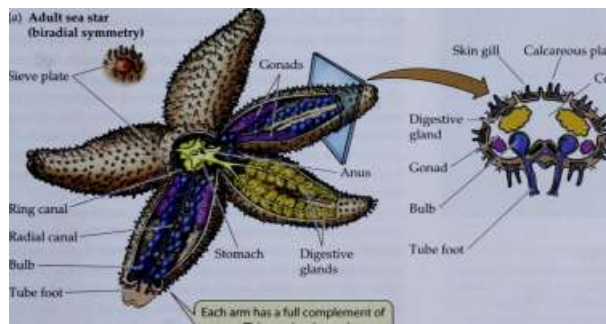
(a) Adult sea star

(biradial symmetry)

Sieve plate

Calcareous plate

Coelom



Tube foot

Each arm has a full complement of organs. This arm has been drawn with the digestive glands removed to show the organs lying below.

(b) Sea star larva

(bilateral symmetry)



The sea star larva moves through the water by beating its cilia.

33.2 Echinoderms Display Two Evolutionary Innovations

This sea star displays the canals and tube feet of the echinoderm water vascular system, as well as a calcified internal skeleton.

580 CHAPTER THIRTY-THREE



(c) Boliadschia argns



(e) *Opiothrix suemsonii*

33.3 Diversity among the Echinoderms

(a) The flexible arms of the golden feather star are clearly visible.

(b) Purple sea urchins are important grazers of algae in the inter-tidal zone of the Pacific Coast of North America, (c) This sea cucumber lives on rocky substrates in seas around Papua New Guinea, (d) The blood sea star is typical of many sea stars; some species, however, have more than five arms, (e) This brittle star is resting on a sponge.

Pelmatozoans have jointed arms

Sea lilies and feather stars (class Crinoidea) are the only surviving pelmatozoans. Sea lilies were abundant 300-500 mya, but only about 80 species survive today. Most sea lilies attach to a substratum by means of a flexible stalk consisting of a stack of calcareous discs. The main body of the animal is a cup-shaped structure that contains a tubular digestive system. Five to several hundred arms, usually in multiples of five, extend outward from the cup. The jointed calcareous plates of the arms enable them to bend. A groove runs down the center of each arm to the mouth. On both sides of the groove are tube feet covered with mucus-secreting glands.

A sea lily feeds by orienting its arms in passing water currents. Food particles strike and stick to the tube feet, which transfer the particles to the grooves in the arms, where the action of cilia carries the food to the mouth. The tube feet of sea lilies are also used for gas exchange and elimination of nitrogenous wastes.

Feather stars are similar to sea lilies, but they have flexible appendages with which they grasp the substratum while they are feeding and resting (Figure 33.3a). Feather

DEUTEROSTOMATE ANIMALS 581

stars feed in much the same manner as sea lilies. They can walk on the tips of their arms or swim by rhythmically beating their arms. About 600 living species of feather stars have been described.

Eleutherozoans are the dominant echinoderms

Most surviving echinoderms are members of the eleuthero-zoan lineage. Biochemical data suggest that the ancestors of sea urchins and sand dollars (class Echinoidea) were the first to split off from the lineage leading to the other eleutherozoans. Sea urchins and sand dollars lack arms, but they share a five-part body plan with all other echinoderms. Sea urchins are hemispherical animals that are covered with spines attached to the underlying skeleton via ball-and-socket joints (Figure 33.3b). The spines of sea urchins come in varied sizes and shapes; a few produce highly toxic substances. Many sea urchins consume algae, which they scrape from the rocks with a complex rasping structure. Others feed on small organic debris that they collect with their tube feet or spines. Sand dollars, which are flattened and disc-shaped, feed on algae and fragments of organic matter on the seafloor.

The tube feet of sea cucumbers (class Holothuroidea; Figure 33.3c) are used primarily for attaching to the substratum rather than for moving. The anterior tube feet are modified into large, feathery, sticky tentacles that can be protruded around the mouth. Periodically, a sea cucumber withdraws the tentacles into its mouth, wipes off the material that has adhered to them, and digests it.

Sea daisies (class Concentricycloidea) were not discovered until 1986. Little is known about them. They have tiny disc-shaped bodies with a ring of marginal spines, and two ring canals, but no arms. Sea daisies are found on rotting wood in ocean waters. They apparently feed on prokaryotes, which they digest outside their bodies and absorb either through a membrane that covers the oral surface or via a shallow saclike stomach.

The most familiar echinoderms are the sea stars (class Asteroidea; Figure 33.3d; see also Figure 33.2). Their tube feet serve as organs of locomotion and, because their walls are thin, they are important sites for gas exchange. Each tube foot of a sea star is also an adhesive organ, consisting of an internal bulb connected by a muscular tube to an external sucker. A tube foot is moved by expansion and contraction of the circular and longitudinal muscles of the tube. It can adhere to a surface by secreting a sticky substance around the sucker.

Many sea stars prey on polychaetes, gastropods, bivalves, and fishes. They are important predators in many marine environments, such as coral reefs and rocky inter-tidal zones. With hundreds of tube feet acting simultaneously, a sea star can exert an enormous and continuous force. It can grasp a clam in its arms, anchor the arms with its tube feet, and, by steady contraction of the muscles in the arms, gradually exhaust the muscles with which the clam keeps its shell closed. Sea stars that feed on bivalves are able to push the stomach out through the mouth and

then through the narrow space between the two halves of the shell. The stomach secretes digestive enzymes into the soft parts of the bivalve, digesting it.

Brittle stars (class Ophiuroidea) are similar in structure to sea stars, but their flexible arms are composed of jointed hard plates (Figure 33.3e). Brittle stars generally have five arms, but each arm may divide a number of times. Most of the 2,000 species of brittle stars ingest particles from the surfaces of sediments and assimilate the organic material from them, but some species remove suspended food particles from the water; others capture small animals. They eject the indigestible particles through their mouths because, unlike most other echinoderms, brittle stars have only one opening to their digestive tract.

Chordates: New Ways of Feeding

The second major lineage of deuterostomes, the phylum Chordata, evolved several different modifications of the coelomic cavity that provided new ways of capturing and handling food. Some living representatives of one early lineage—acorn worms—live buried in marine sand or mud, under rocks, or attached to algae. They may be similar to the ancestors of the chordate lineage, but are currently classed in their own lineage as hemichordates ("half-chor-dates"). Animals in the chordate lineage evolved a strikingly different body plan from the acorn worms, characterized by an internal dorsal supporting structure, which in the vertebrates evolved into the spinal column.

Acorn worms capture prey with a proboscis

The acorn worms (phylum Hemichordata) have a three-part body consisting

of a proboscis, collar, and trunk (Figure 33.4). The 70 species of acorn worms live in burrows in muddy and sandy sediments.

The large proboscis of acorn vertebrata

worms is a digging organ. It is coated with a sticky mucus that traps prey items in the sediment. The mucus and its attached prey are conveyed by cilia to the mouth. In the esophagus, the food-laden mucus is compacted into a ropelike mass that is moved through the digestive tract by ciliary action. Behind the mouth is a pharynx that opens to the outside through a number of pharyngeal slits through which water can exit. Highly vascularized tissue surrounding the pharyngeal slits serves as a gas exchange apparatus. An acorn worm breathes by pumping water into its mouth and out through its pharyngeal slits.

The pharynx becomes a feeding device

The same property required for effective gas exchange— a large surface area—also serves well for capturing prey. The pharyngeal slits, which originally functioned as sites for

Echinodermata



582 CHAPTER THIRTY-THREE

Trunk

Collar

Proboscis



Saccoglossus kowalewski

33.4 A Hemichordate

The proboscis (right) of this acorn worm is modified for digging. This individual has been extracted from its burrow.



33.5 Tunicates

Pharyngeal baskets occupy most of the body cavities of these transparent sea squirts. The blue color is a reflection of the environment in this photograph.

gas exchange and eliminating water, as they do in modern acorn worms, were enlarged in a sister lineage. This enlargement of the pharyngeal slits eventually led to the remarkable evolutionary developments that gave rise to the chordates.

Chordates (phylum Chordata) are bilaterally symmetrical animals whose body plans are characterized by several shared features:

- ▶ Pharyngeal slits (at some stage of their development).
- ▶ A dorsal, hollow nerve cord.
- ▶ A ventral heart.
- ▶ A tail that extends beyond the anus.
- ▶ A dorsal supporting rod, the notochord.

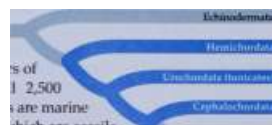
The notochord is the most important derived trait of the Chordata and is unique to that phylum. In some species, such as tunicates, the notochord is lost during metamorphosis to the adult stage. In the vertebrates, it is replaced by skeletal structures that provide support for the body.

A notochord appears in tunicates and lancelets

The tunicates (sub-phylum Urochordata) may be similar to the ancestors of all chordates. All 2,500 species of tunicates are marine animals, most of which are sessile as adults. It is their swimming, tadpolelike larvae that reveal the close evolutionary relationships between tunicates and other chordates.

A tunicate larva has pharyngeal slits, a dorsal, hollow nerve cord, and a notochord. Muscles are attached to the notochord, providing the body with relatively rigid support. After a short time floating in the water, the larva settles on the seafloor and becomes a sessile adult. The nerve cord and notochord disappear in the adult animal, which

Echinodermata



Vertebra ta

feeds by extracting plankton from the water. An adult's pharynx is enlarged into a pharyngeal basket lined with cilia, whose beating moves water through the animal.

More than 90 percent of known species of tunicates are sea squirts (class Ascidiacea). Some sea squirts are solitary,

Mouth

Pharyngeal basketXT

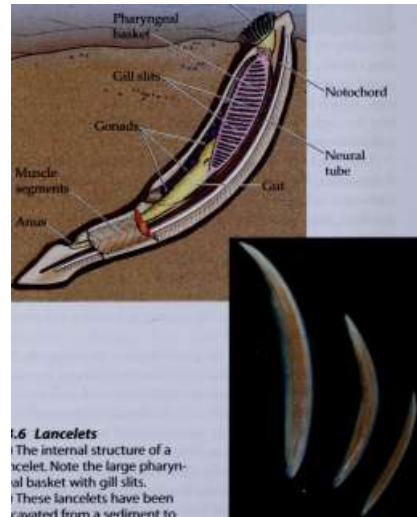
Gill slits

Notochord

Muscle segments

Anus

(fl)

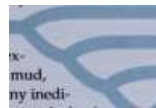


33.6 Lancelets

(a) The internal structure of a lancelet. Note the large pharyngeal basket with gill slits. (b) These lancelets have been excavated from a sediment to show the entire body.

(b) *Branchiostoma californiense*

DEUTEROSTOMATE ANIMALS 583



Echinodermata

Hemichordata

Cephalochordata

but others produce colonies by asexual budding from a single founder. Individual sea squirts range in size from less than 1 mm to 60 cm in length, but colonies may measure several meters across. The baglike body of an adult is surrounded by a tough tunic, composed of protein and a complex polysaccharide, which is secreted by the epidermal cells. Much of the body is occupied by the large pharyngeal basket (Figure 33.5).

The 25 species of lancelets (subphylum Cephalochordata) are small animals that rarely exceed 5 cm in length. Their notochord extends the entire length of the body throughout their lives, and they resemble small fishes. Lancelets live partly buried in soft marine sediments. They extract small prey from the water with their pharyngeal baskets (Figure 33.6).

Origin of the Vertebrates

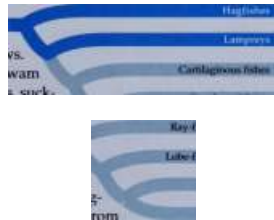
In one chordate lineage, the pharyngeal basket became enlarged. With its many exit openings, an enlarged basket was effective in extracting prey from mud, which contains many inedible particles along with food. This lineage gave rise to the vertebrates (subphylum Vertebrata). In the late Cambrian period, these early vertebrates evolved improved structures for extracting food from mud and sand and for moving over the surface of the substratum.

Vertebrates take their name from a jointed, dorsal vertebral column, which replaced the notochord as their primary support. The vertebrate body plan (Figure 33.7) can be characterized as follows:

- ▶ With the vertebral column as its anchor, a rigid internal skeleton provides support and mobility.
- ▶ Two pairs of appendages are attached to the vertebral column.
- ▶ The faster locomotion made possible by appendages favored the evolution of an anterior skull with a large brain and highly developed sensory receptors.
- ▶ The internal organs are suspended in a large coelom.
- ▶ A well-developed circulatory system, driven by contractions of a ventral heart, delivers oxygen to internal organs.

The filter-feeding ancestral vertebrates lacked jaws. They probably swam over the sediments, sucking up mud and extracting microscopic food from it. These animals gave rise to the fishes. The lineage leading to modern hagfishes probably separated first from

The anterior skull contains the brain and many sensory organs.



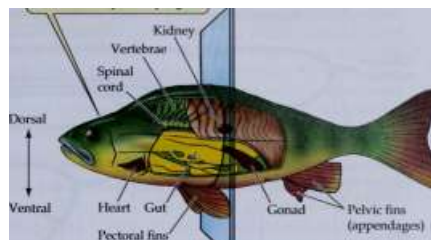
Cartilaginous fishes

Ray-finned fishes

Lobe-finned fishes

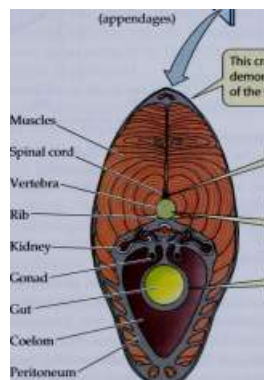
Lungfishes

Tetrapods



Pectoral fins (appendages)

Pelvic fins (appendages)



This cross section of a bony fish demonstrates some key elements of the vertebrate body plan, including:

A dorsal nervous system...

...an internal skeleton, centered on the vertebral column...

...and organs suspended in the coelom.

Coelom Peritoneum

33.7 The Vertebrate Body Plan

A bony fish is used here to illustrate the structural elements common to all vertebrates.

the other groups (Figure 33.8). One early group of jawless fishes, called ostracoderms, meaning "shell-skinned," evolved a bony external armor that protected them from predators. With their heavy armor, these small fishes could swim only slowly, but they could safely swim above the substratum, which was easier than having to burrow through it, as all previous sediment feeders had done.

The new mobility of jawless fishes enabled them to exploit their environments in new ways. They could attach to dead organisms and use the pharynx to create suction to pull fluids and partly decomposed tissues into the mouth. Hag-fishes and lampreys, the only jawless fishes to survive beyond the Devonian period, feed on both dead and living organisms in this way (Figure 33.9). These fishes have tough, scaly skins instead of external armor. The round mouth is a sucking organ with which the animals attach to their prey and rasp at the flesh. Lampreys live in both fresh and salt water; many species move between the two environments, laying their eggs in rivers and maturing in the sea.

Hagfishes have become entirely marine...

...but lampreys live in marine, freshwater, and estuarine environments

Common ancestor

Marine and freshwater vertebrates are thought to have developed from estuarine ancestors.

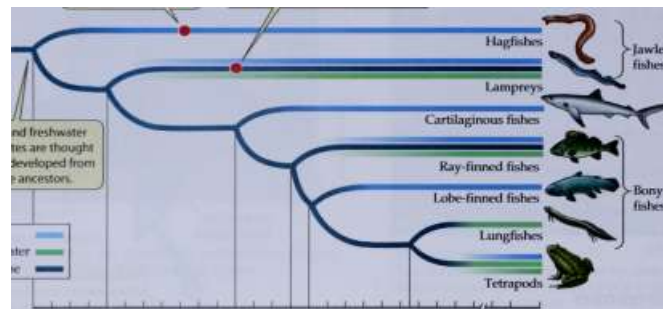
Key

Marine

Freshwater

Estuarine

Mawless I fishes



550

500

450 400

Millions of years ago (mya)

33.8 A Probable Vertebrate Phylogeny

This phylogeny incorporates the view that vertebrates evolved in estuaries, where their ability to handle varying salinities allowed them to exploit habitats not available to marine animals.

Jaws improve nutrition

During the Devonian period, many new kinds of fishes evolved in the seas, estuaries, and fresh waters. Although most of these were jawless, members of one lineage evolved jaws from some of the skeletal arches that supported the gill region (Figure 33.10). A jaw allows a fish to grasp and subdue relatively large, living prey. Further development of jaws and teeth among fishes led to the ability to chew both soft and hard body parts of prey. Chewing aided chemical digestion and improved the ability of fishes to obtain nutrients from prey.

350

Present



Petromyzon marinus

33.9 A Modern Jawless Fish

A lamprey uses its large, jawless mouth to suck blood and flesh from other fishes.

The dominant early jawed fishes were the heavily armored placoderms (class Placodermi). Some of these fishes evolved elaborate appendages and relatively sleek body forms that improved their ability to maneuver in open water. A few became huge (10 meters long) and, together with squids (cephalopod mollusks), were probably the major predators in the Devonian oceans. Despite their early abundance, however, most placoderms disappeared by the end of the Devonian period; none survived to the end of the Paleozoic era.

Fins improve mobility

Two other groups of fishes—the bony fishes and the cartilaginous fishes, both ^^^^^^^^^^^^^^^^^

r , • , _ ^tf Hagfishes

of which survive

today—became Lampreys

abundant during the Devonian period.

Cartilaginous fishes Ray-finned fishes

(class Chondrichthyes)—

the sharks, skates and rays, and chimaeras (Figure 33.11)— Lungfishes

have a skeleton composed entirely ^^

of a firm but pliable material called Tetrapods

cartilage. Their skin is flexible and leathery, sometimes bearing bristly projections that give it the consistency of sandpaper. The loss of external armor increased their mobility and ability to escape from predators.

In the cartilaginous fishes and their descendants, swimming is controlled by pairs of unjointed appendages called fins: a pair of pectoral fins just behind the gill slits and a pair of pelvic fins just in front of the anal region. A dorsal median fin stabilizes the fish as it moves. Sharks move forward by means of their tail and pelvic fins. Skates and rays propel themselves by means of the undulating movements of their greatly enlarged pectoral fins.

Cartilaginous fishes

Lobe-finned fishes

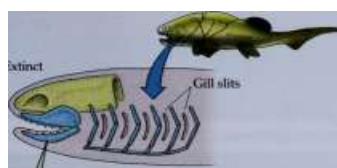
Jawless fishes (agnathans) Extinct and living forms



Gill arches are made of cartilage.

Early jawed fishes (placoderms)

Extinct



Some anterior gill arches became modified to form jaws.

Modern jawed fishes (cartilaginous and bony fishes)

Living forms



Additional gill arches were incorporated to form heavier, more efficient jaws.

33.10 Jaws from Gill Arches

This illustrates one probable scenario for the evolution of jaws from the anterior gill arches of fishes.

(a) *Triaenodon obesus*

Most sharks are predators, but some feed by filtering plankton from the water. The world's largest fish, the whale shark (*Rhincodon typhus*), is a filter feeder. It may grow to more than 15 meters in length and weigh more than 9,000 kilograms. Most skates and rays live on the ocean floor, where they feed on mollusks and other invertebrates buried in the sediments. Nearly all cartilaginous fishes live in the oceans.

Swim bladders allow control of buoyancy

The bony fishes (class Osteichthyes) have internal skeletons of bone rather than cartilage, giving them their common name. Their bony skeleton is lighter than that of the cartilaginous fishes. In most species, the outer surface is covered with flat, smooth, thin, lightweight scales that provide some protection. The gills of bony fishes open into a single chamber cov- _____

A U U A Hagfishes

ered by a hard

flap. Movement Lampreys

of the flap improves the flow of water over the gills, where gas exchange takes place.

Early bony fishes also evolved gas-filled sacs that supplemented the action of the gills in respiration. These features enabled po< ^

early bony fishes to live where oxygen was periodically in short supply, as it often is in estuarine and freshwater environments. They still serve this function in lungfishes and a few other modern fishes, but in the ray-finned fishes— a group that includes most of the many species of bony fish—these lunglike sacs evolved into swim bladders,

Cartilaginous fishes

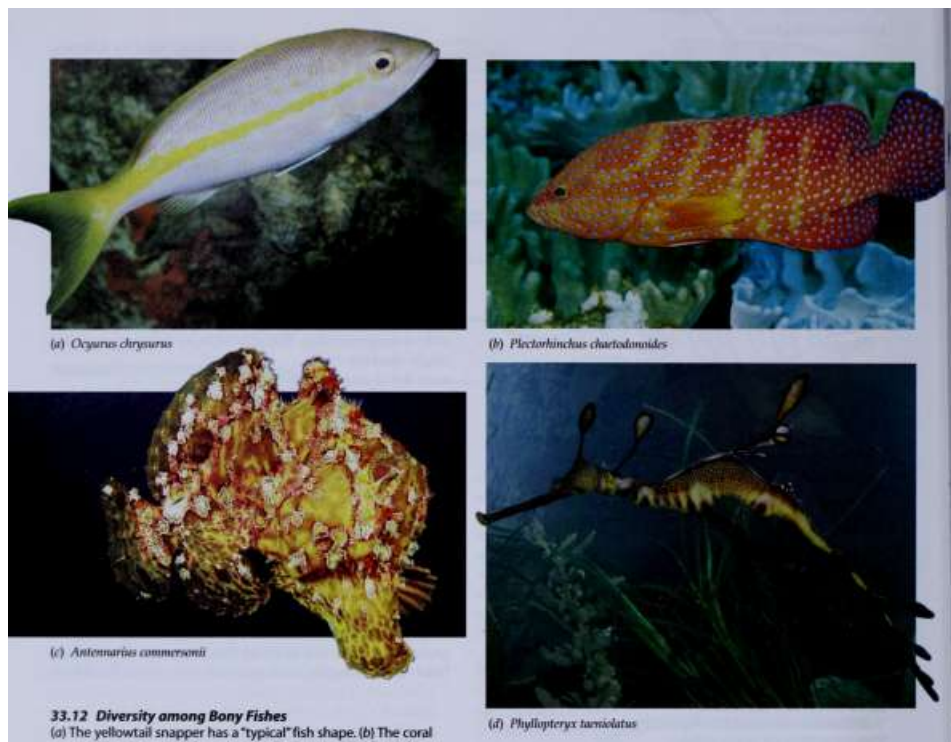


33.11 Cartilaginous Fishes

(a) Most sharks, such as this whitetip reef shark, are active marine predators, (b) Skates and rays, represented here by a stingray, feed on the ocean bottom. Their modified pectoral fins are used for propulsion.

(b) Trygon pastinaca





(c) *Antennarius commersonii*

33.12 Diversity among Bony Fishes

(a) The yellowtail snapper has a "typical" fish shape, (b) The coral grouper lives on tropical coral reefs, (c) Commerson's frogfish can change its color in a range from pale yellow to orange-brown to deep red, thus enhancing its camouflage abilities, (d) This weedy sea dragon is difficult to see when it hides in vegetation. It is a larger "cousin" of the more familiar seahorse.

which serve as organs of buoyancy. By adjusting the amount of gas in its swim bladder, a fish can control the depth at which it is suspended in the water without expending energy.

With their lighter skeletons and protective coverings and their swim bladders, ray-finned bony fishes evolved a remarkable diversity of sizes, shapes, and lifestyles (Figure 33.12). The smallest are less than 1 cm long as adults; the largest are ocean sunfishes that weigh up to 900 kilograms. Ray-finned fishes exploit nearly all types of aquatic food sources. In the oceans they filter plankton from the water, rasp algae from rocks, eat corals and other colonial invertebrates, dig invertebrates from soft sediments, and prey on virtually all other vertebrates except large whales and Ichthyosaurs. In fresh water they eat plankton, devour insects

(d) *Phyllopteryx taeniolatus*

of all aquatic orders, eat fruits that fall into the water in flooded forests, and prey on other aquatic vertebrates.

Some fishes live buried in soft sediments, capturing passing prey or emerging at night to feed. Many are solitary, but in open water others form large aggregations called schools. Many fishes perform complicated behaviors by means of which they maintain schools, build nests, court and choose mates, and care for their young.

With their fins and swim bladders, fishes can readily control their positions in open water, but their eggs tend to sink. Therefore, most fishes attach their eggs to the substratum, although a few species discharge their small eggs directly into surface waters where they are buoyant enough to complete their development before they sink very far. Most marine fishes move to food-rich shallow waters to lay their eggs, which is why coastal waters and estuaries are so important in the life cycles of many species. Some, such as salmon, abandon salt water when they breed, ascending rivers to spawn in freshwater streams and lakes.

DEUTEROSTOMATE ANIMALS 587

Colonizing the Land: Obtaining Oxygen from the Air

Although the evolution of lunglike sacs was a response to the inadequacy of gills for respiration in oxygen-poor waters, it also set the stage for the invasion of land. Some early bony fishes probably used their lung sacs to supplement their gills when oxygen levels in the water were low. This ability would also have allowed them to breathe air, and to leave the water temporarily when pursued by predators unable to do so. But with their unjointed fins, bony fishes could only flop around on land, as most fishes do today if placed out of water. Changes in the structure of fins would help such fishes move about on land.

Two lineages of bony fishes evolved jointed fins: the lobe-finned fishes (subclass *Crossopterygii*) and the lung-fishes. The lobe-fins flourished from the Devonian period until about 25 mya, when they were thought to have become extinct. However, in 1938, a lobe-fin was caught by commercial fishermen off the Comoro Islands in the Indian Ocean. Since that time, several

dozen specimens of this extraordinary fish, *Latimeria chalumnae*, have been collected. *Latimeria*, a predator on other fishes, reaches a length of about 1.5 meters and weighs up to 82 kilograms (Figure 33.13). Although it belongs to the bony fish lineage, the skeleton of *Latimeria* is composed mostly of cartilage, not bone.

Some descendants of early fishes with jointed fins began to use terrestrial food sources and over time became more fully adapted to life on land. This lineage became the tetra-pods: the four-legged amphibians, reptiles, birds, and mammals that are common today.

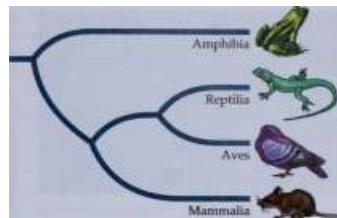
Amphibians invade the land

During the Devonian period, amphibians (class Amphibia) arose from ancestors they shared with the lung-fishes. In this lineage, the stubby, jointed fins of their ancestors evolved into walking legs. The design of those legs has

Tetrapod

vertebrate

ancestor



33.73 A Modern Lobe-Fin

Latimeria chalumnae, found in deep waters of the Indian Ocean, is the sole surviving species of its lineage, which had been thought to be extinct.

33.14 A Probable Phytogeny of Tetrapods

In the birds (Class Aves), the paired forelimbs evolved into wings.

remained largely unchanged throughout the evolution of terrestrial vertebrates (Figure 33.14).

The Devonian predecessors of amphibians were probably able to crawl from one pond or stream to another by pulling themselves along on their finlike legs, as do some modern species of catfishes. They gradually evolved to be able to live on swampy land and, eventually, on dry land. Living amphibians have relatively small lungs, and most species exchange gases through their skins. Most terrestrial species are confined to moist environments because they lose water rapidly through their skins when exposed to dry air.

About 4,500 species of amphibians live on Reptilia

Earth today, many fewer than the number known only from fossils. Living amphibians belong to three orders (Figure 33.15):

the wormlike, tropical, burrowing caecilians (order Gymno-phiona); frogs and toads (order Anura, which means "tailless"); and salamanders (order Urodela, which means "tailed"). Most species of frogs and toads live in tropical and warm temperate regions, although a few are found at very high latitudes and altitudes. Salamanders are more diverse in temperate regions, but many species are found in cool, moist environments in the mountains of Central America.

Most species of amphibians live in water at some time in their lives. In the typical amphibian life cycle, part or all of the adult stage is spent on land, usually in a moist habitat, but adults return to fresh water to lay their eggs (Figure 33.16). An amphibian egg can survive only in a moist environment because it is enclosed by a delicate envelope that cannot prevent water loss in dry conditions. The fertilized eggs of most species give rise to larvae that live in water until they change into terrestrial adults.

Amphibians are the focus of much attention today because populations of many species are declining rapidly. The golden

toad, for example, has disappeared from the Monteverde Cloud Forest Reserve in Costa Rica, which was established primarily to protect this rare species. Several possible reasons for the declines, including drought, in-

588 CHAPTER THIRTY-THREE

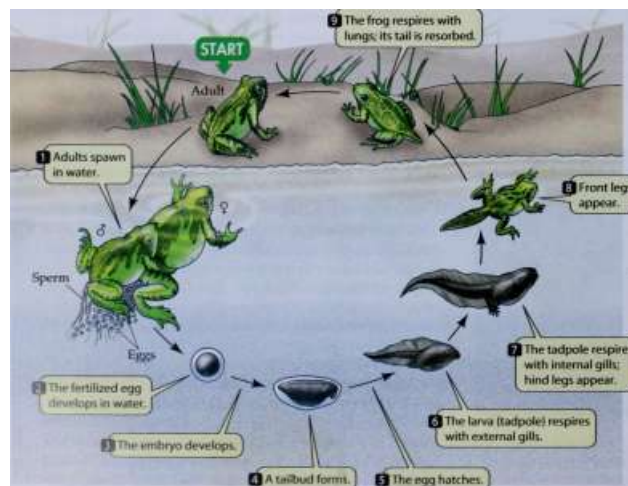


(b) *Scaphiophryne gottlebei*

(c) *Salamandra salamandra*

creased ultraviolet radiation, and diseases, have been identified. Biologists are monitoring amphibian populations closely to learn more about the causes of their difficulties and to determine the implications of their declines for other organisms, including humans.

The frog respire with lungs; its tail is resorbed.



33.15 Diversity among the Amphibians

- (a) Burrowing caecilians superficially look more like worms than amphibians.
- (b) A rare frog species discovered in a national park on the island of Madagascar.
- (c) A European fire salamander.

Amniotes colonize dry environments

Most amphibians, as we have just seen, are limited to moist environments. Two morphological changes contributed to the ability of one lineage of vertebrates to control water loss and, therefore, to exploit a wider range of terrestrial habitats. One was the evolution of an egg with a shell that is relatively impermeable to water. The other was a combination of traits that

reduced water loss, including a tough skin impermeable to water and kidneys that could excrete concentrated urine. The vertebrates that evolved both of these traits are called amniotes. They were the first vertebrates to become widely distributed over the terrestrial surface of Earth.

The amniote egg has a leathery or brittle calcium-impregnated shell that retards evaporation of the fluids inside, but permits O_2 and CO_2 to pass through. Such an egg

does not require a moist environment, but can be laid anywhere. Within the shell and surrounding the embryo are membranes that protect the embryo from desiccation and assist its respiration and excretion of waste nitrogen. The egg also stores large quantities of food as yolk, permitting the embryo to attain a relatively advanced state of development before it hatches and *vs.* must feed itself (Figure 33.17).

An early amniote lineage, the reptiles (class Reptilia) arose from the tetrapods during the Carboniferous period. As we discussed in Chapter 23, Rep-

dpole respire with internal gills; hind legs appear.

The larva (tadpole) respire with external gills.



The egg hatches.

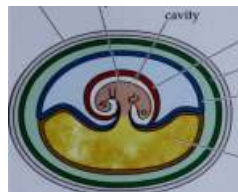
33.16 In and Out of the Water

Most stages in the life cycle of temperate-zone frogs take place in water. The aquatic tadpole is transformed into a terrestrial adult through metamorphosis.

Shell

Embryo

Amniotic cavity



Amnion

A

Chorion >

Allantois

Extraembryonic membranes



Yolk

33.17 An Egg for Dry Places

The evolution of the amniote egg, with its shell, three extraembryonic membranes and embryo-nourishing yolk, was a major step in the colonization of the terrestrial environment.

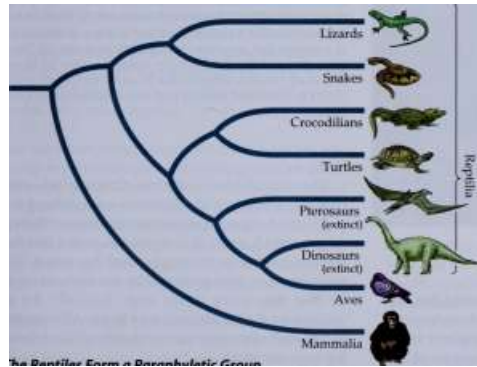
tilia, as we use the term here, is a paraphyletic group because it does not include the birds, a major lineage that split off relatively recently during reptilian evolution. (Figure 33.18). However, because all reptiles are structurally similar, they serve as a convenient example for discussing the characteristics of amniotes. Therefore, we use this traditional classification as a basis for our discussion while recognizing that the birds should technically be included within it.

About 6,000 species of reptiles live today. Most reptiles do not care for their eggs after laying them. In some species the eggs do not develop shells, but are retained inside the female's body until they hatch. Still other species evolved structures called placentas that nourish the developing embryos.

Amphibia

Aves

Mammalia



33.18 The Reptiles Form a Paraphyletic Group

The traditional classification of the reptiles creates the paraphyletic group Reptilia. As used here, Reptilia does not include the birds (Aves), even though this major lineage split off from the crocodilian reptiles relatively recently (in evolutionary terms).

The skin of a reptile is covered with horny scales that greatly reduce loss of water from the body surface. These scales, however, make the skin unavailable as an organ of gas exchange. In reptiles, gases are exchanged almost entirely by the lungs, which are proportionally much larger in surface area than those of amphibians. A reptile forces air into and out of its lungs by bellows-like movements of its ribs. Unlike the amphibian heart, the reptilian heart is divided into chambers that partially separate oxygenated from unoxygenated blood. With this type of heart, reptiles can generate higher blood pressures than amphibians and can sustain higher levels of muscular activity.

Reptilian lineages diverge

The lineages leading to modern reptiles began to diverge about 250 mya when the ancestors of the subclass Squamata (lizards, snakes, and amphisbaenians—a group of legless, wormlike, burrowing animals with greatly reduced eyes) diverged from the lineage leading to all other reptiles (Figure 33.19a, b). Most lizards are insectivores, but some are herbivores, and a few prey upon other vertebrates. The largest lizards, which may grow as long as 3 meters, are some species of monitors that live in the East Indies. Most lizards walk on four limbs, but some are limbless, as are all snakes, which are descendants of burrowing lizards.

All snakes are carnivores, and many can swallow objects

much larger in diameter than themselves. The combination

of poison glands and the ability to inject venom rapidly

into their prey evolved several times. The largest snakes are

pythons more than 10 meters long.

The tuataras (subclass Sphenodontida), which today are represented by only two species restricted to a few islands off the coast of New Zealand (Figure 33.19c), superficially resemble lizards, but differ from them in several internal anatomical features. Their phylogenetic relationships are uncertain.

Considerable uncertainty surrounds the next lineage split. Traditionally turtles were thought to have separated from other reptiles early in the history of the group, but new molecular analyses suggest that turtles are closely related to crocodilians, which diverged later. Both turtles and ancestral crocodiles have dorsal and ventral armored plates, and such plates have characterized those groups for many millions of years.

The dorsal and ventral bony plates of modern turtles and tortoises (subclass Chelonia) form a shell into which the head and limbs can be withdrawn (Fig-



(b) *Ocyurus chrysurus*



(c) *Sphenodon punctatus*

ure 33.19d). Most turtles live in lakes and ponds, but tortoises are terrestrial; some live in deserts. Sea turtles spend their entire lives at sea except when they come ashore to lay eggs; all seven species are endangered. A few species of turtles and tortoises are carnivores, but most species are omnivores that eat a variety of aquatic and terrestrial plants and animals.

(e) *Alligator mississippiensis*

Figure 33.19 Reptilian Diversity

(a) This Sumatran pit viper is prepared to strike, (b) This African chameleon, a lizard, has a long tail with which it can grasp branches and large eyes that move independently in their sockets, (c) The tuatara looks like a typical lizard, but it is one of only two survivors of a lineage that separated from lizards long ago. (d) The green sea turtle is widely distributed in tropical oceans, (e) Most crocodilians are tropical; alligators live in warm temperate environments in China and, like this one, in the southeastern United States.

The crocodilians (subclass Crocodylia)—crocodiles, caimans, gharials, and alligators—are confined to tropical and warm temperate environments (Figure 33A9e). Crocodilians spend much of their time in water, but they build nests on land or on floating piles of vegetation. Their eggs are warmed by heat generated by the decay of organic matter that they place in the nest. Typically the eggs are guarded by the female until they hatch. All crocodilians are carnivorous; they prey on vertebrates of all classes, including large mammals.

Another lineage led to the dinosaurs, reptiles that rose to dominance about 215 mya and dominated terrestrial environments for about 150 million years. During this time, vir-

Amphibia

Reptilia

Mammalia

tually all terrestrial animals more than 1 meter in length were dinosaurs. Some of the largest dinosaurs weighed up to 100 tons. Many were agile and could run rapidly. Some small predatory dinosaurs evolved feathers.

The ability to breathe and run simultaneously, which we take for granted, was a major innovation in the evolution of terrestrial vertebrates. Not until the evolution of the lineages leading to the mammals, dinosaurs, and birds did the legs assume vertical positions, which reduced the lateral forces on the body during locomotion. Special ventilatory muscles that

enabled the lungs to be filled and emptied while the limbs moved also evolved. These muscles are visible in living birds and mammals; we can infer their existence in dinosaurs from the structure of the vertebral column in their fossils and the capacity of many dinosaurs for bounding bipedal (using two legs) locomotion.

Birds: Feathers and Flight

During the Mesozoic era, a dinosaur lineage gave rise to the birds (subclass Aves). The oldest known avian fossil, Archaeopteryx, which lived ^^^^^^ about 150 mya, was covered with feathers that are virtually identical to those of modern birds. It also had well-developed wings, a long tail (Figure 33.20a), and a wishbone, which in modern birds serves as an anchoring site for flight muscles. Archaeopteryx had typical perching bird claws, suggesting that it lived in trees and shrubs and used the clawed fingers on its forearms to assist it in clambering over branches.

Another early bird, Confuciusornis sanctus, which is known from hundreds of complete fossils from China, lived only slightly more recently than Archaeopteryx. Well-preserved fossils show that males had greatly elongated tail feathers (Figure 33.20b), which they probably used in communal courtship displays. Large numbers of these fossils have been found together, as would be expected if a number of males displayed together on communal display grounds.

Because the avian lineage separated from the other reptiles long before Archaeopteryx lived, existing data are insufficient to identify the ancestors of birds with certainty. Most paleontologists (scientists who study fossils) believe that birds evolved from terrestrial bipedal dinosaurs that used their forelimbs for capturing prey. According to this view, these small dinosaurs evolved feathers for insulation or display, and eventually were able to become airborne for short distances.

During the Cretaceous period, birds underwent an extensive evolutionary radiation. The dominant Cretaceous lineage was the "opposite birds," so named because the tarsal bones of their legs fused in the opposite direction from the way fusion happens in all modern birds. All lineages of opposite birds died out at the end of the Cretaceous,



33.20 Mesozoic Birds

(a) An artist's recreation of Archaeopteryx shows its modern feathers, arboreal habits, and flight. (fc>) The elongated tail feathers of a male Confuciusornis sanctus ("sacred bird of Confucius") fossil suggest that the males used them in courtship displays.

but scientists disagree over how many other avian lineages survived the mass extinction. Paleontologists believe that members of only one lineage, collectively known as the transitional shorebirds, survived the end of the Cretaceous, because no later fossils of other lineages have been found. Other scientists, who base their conclusions on molecular clocks, believe that at least some representatives of many Cretaceous avian lineages must have survived, because modern birds are too different to have come from a single lineage.



33.21 Diversity among the Birds

(a) Penguins such as these gentoos are widespread in the cold waters of the southern hemisphere. They are expert swimmers, although they have lost the ability to fly. (b) Parrots are a diverse group of birds, especially in the tropics of Asia and the Pacific islands. This Australian king parrot is one member of Australia's rich parrot fauna, (c) Perching birds, represented here by a male northern cardinal, are the most species-rich of all the bird lineages.

(a) *Pygoscelis papua*



■ HHHBHHHBHHBHSIMi

(b) *Alisterus scapularis*

As a group, birds eat almost all types of animal and plant material. A few aquatic species have bills modified for filtering small food particles from the water. Insects are the most important dietary item for terrestrial birds. In addition, they eat fruits and seeds, nectar and pollen, leaves and buds, carrion, and other vertebrates. Birds are major predators of flying insects during the day, and some species exploit that food source at night. By eating the fruits and seeds of plants, birds serve as major agents of seed dispersal.

The feathers developed by some dinosaurs may originally have had thermoregulatory or display functions. Birds use them for these purposes as well as for flying. The flying surface of the wings is created by large quills that arise from the forelimbs. Other strong feathers sprout like a fan from the shortened tail and serve as stabilizers during flight. The contour feathers and down feathers provide insulation to control loss of body heat.

The bones of birds are also modified for flight. They are hollow and have internal struts for strength. The sternum as a bone forms a large, vertical keel to which the breast muscles are attached. These muscles pull the wings downward during the main propulsive movement in flight.

(c) *Cardinalis cardinalis*

Flight is metabolically expensive, and a flying bird consumes energy at a very high rate—about eight times the amount of energy per day as a lizard of the same weight! Because birds have such high metabolic rates, they generate large amounts of heat. They control the rate of heat loss using their feathers, which may be held close to the body or elevated to alter the amount of air trapped as insulation. The brain of a bird is larger in proportion to its body size than lizard or crocodile brains, primarily because the cerebellum, the center of sight and muscular coordination, is enlarged. The beaks of modern birds lack teeth.

Most birds lay their eggs in a nest, where they are warmed by the body heat of an adult that sits on them. Because birds have high body temperatures, the eggs of most species develop rapidly, hatching in less than 2 weeks. The offspring of many species hatch at a relatively helpless stage and are fed for some time by their parents. The young of other bird species, such

as chickens, sandpipers, and ducks, can feed themselves shortly after hatching. Adults of all species attend their offspring for some time, warning them of and protecting them from predators, leading them to good foraging places, sheltering them from bad weather, and feeding them.

As adults, birds range in size from the 2-gram bee hummingbird of the West Indies to the 150-kg ostrich. Some flightless birds of Madagascar and New Zealand known from fossils were even larger, but they were exterminated by the humans that first colonized those islands. There are about 9,600 species of living birds, more than in any other major vertebrate group except fishes (Figure 33.21).

Amphibia

Aves



The Origin and Diversity of Mammals

Mammals (class Mammalia) appeared in the early part of the Mesozoic era, branching from a lineage of mammal-like reptiles. Small mammals coexisted with reptiles and dinosaurs for at least

150 million years. After the large reptiles and dinosaurs disappeared during the mass extinction at the close of the Mesozoic, mammals increased dramatically in numbers, diversity, and size.

Skeletal simplification accompanied the evolution of small mammals from their larger reptilian ancestors. During mammalian evolution, bones from the lower jaw were incorporated into the middle ear, leaving a single bone in the lower jaw, and the number of bones in the skull decreased. The bulk of both the limbs and the bony girdles from which they are suspended was reduced. Mammals have far fewer, but more highly differentiated teeth than reptiles. Differences in the number, type, and arrangement of teeth in mammals reflect their varied diets.

These skeletal features are readily preserved as fossils, but the soft parts of mammals are seldom fossilized. Therefore we do not know when mammalian features such as mammary glands, sweat glands, hair, and a four-chambered heart evolved. As is the case with birds, the mammalian fossil record suggests that most of the modern mammalian orders evolved rapidly after the end of the Cretaceous, whereas molecular data suggest that they had already evolved during the Cretaceous.

Mammals are unique among animals in providing their young with a nutritive fluid (milk) secreted by mammary glands. Mammalian eggs are fertilized within the female's body, and the embryos undergo some development within a uterus prior to being born. In addition, mammals have a protective and insulating covering of hair, which is luxuriant in some species but has been almost entirely lost in whales, dolphins, and humans. In whales and dolphins thick layers of insulating fat (blubber) replace hair. Clothing assumes the same role for humans.

Mammals range in size from tiny shrews weighing only about 2 grams to the endangered blue whale, which measures up to 31 meters long and weighs up to 160,000 kilograms—the largest animal ever to live on Earth. The approximately 4,000 species of living mammals are divided into two major subclasses: Prototheria and Theria.

The subclass Prototheria contains a single order, the Monotremata, with only three species, which are found only in Australia and New Guinea. These mammals, the duck-billed platypus and spiny anteaters, or echidnas, differ from other mammals in that they lack a placenta, lay eggs, and have legs that poke out to the side (Figure 33.22). Monotremes nurse their young on milk, but they have no nipples on their mammary glands; rather, the milk simply oozes out and is lapped off the fur by the offspring.



(a) *Tachyglossus aculeata*



(b) *Ornithorhynchus anatinus*

33.22 Monotremes

{a) The short-beaked echidna is one of two surviving species of echidnas. (fc») The duck-billed platypus is the third surviving monotreme species.

Two major groups of mammals are members of the subclass Theria. Females of one group, the Marsupialia, have a ventral pouch in which they carry and feed their offspring (Figure 33.23fl). Gestation (pregnancy) in marsupials is short; the young are born tiny but with well-developed fore-limbs, with which they climb to the pouch. They attach to a nipple but cannot suck. The mother ejects milk into the tiny offspring until they grow large enough to suckle. Once her offspring have left the uterus, a female marsupial may become sexually receptive again. She can then carry fertilized eggs capable of initiating development and replacing the offspring in the pouch should something happen to them.

There are about 240 living species of marsupials. At one time marsupials were widely distributed on Earth, but today the majority of species are restricted to the Australian region, with a modest representation in South America

594 CHAPTER THIRTY-THREE



33.23 Marsupials

(a) Australia's kangaroos are thought of as the typical marsupial, but the marsupial radiation also produced (b) arboreal species such as this South American opossum and (c) carnivores such as the Tasmanian devil.

(a) *Macropus rufus*

(b) *Caluromys phicander*

(Figure 33.23b). Only one species, the Virginia opossum, is widely distributed in the United States. Marsupials radiated to become terrestrial herbivores, insectivores, and carnivores, but no species live in the oceans or can fly, although some are gliders. The largest living marsupial is the red kangaroo of Australia, which weighs up to 90 kilo-

33.24 Diversity among the Eutherians

(a) The Arctic ground squirrel is one of many species of small, diurnal rodents of western North America, (b) Temperate-zone bats are all insectivores, but many tropical bats such as this leaf-nosed bat eat fruit, (c) Dolphins represent a eutherian lineage that returned to the marine environment, (d) Large hoofed mammals are important herbivores over much of Earth. This caribou bull is grazing by himself, although caribou are often seen in huge herds.



Late Cretaceous Paleocene Eocene Oligocene Miocene

A

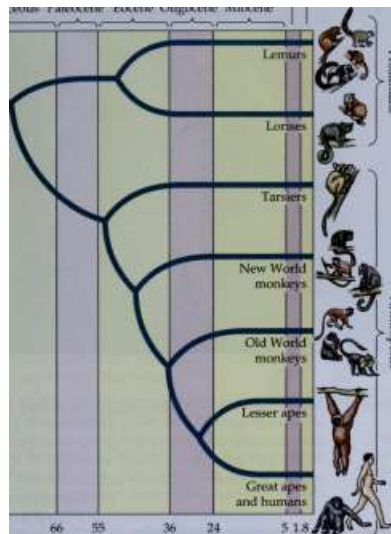
Pliocene "Pleistocene

Ancestral

arboreal

insectivore

98



Millions of years ago (mya)

33.25 A Probable Primate Phylogeny

Too few fossil primates have been discovered to reveal with certainty their evolutionary relationships, but this phylogenetic tree is consistent with existing evidence.

grams, but much larger marsupials existed in Australia until they were exterminated by humans soon after they reached Australia about 50,000 years ago.

Most living mammals are eutherians. (Eutherians are sometimes called placental mammals, but this name is not accurate because some marsupials also have placentas.) Eutherians are more highly developed at birth than are marsupials, and no external pouch houses them after birth. The nearly 4,000 species of eutherians are divided into 16 major groups, the largest of which is the rodents, with about 1,700 species (Figure 33.24f). The next largest group, the bats, has about 850 species (Figure 33.24b), followed by the insectivores (moles and shrews) with slightly more than 400 species.

Eutherians are extremely varied in form and ecology. Several lineages of terrestrial mammals subsequently colonized marine environments, to become whales, dolphins, seals, and sea lions (Figure 33.24c). Eutherian mammals are—or were, until they were greatly reduced in numbers by humans—the most important grazers and browsers in most terrestrial ecosystems

(Figure 33.24d). Grazing and browsing have been an evolutionary force intense enough to select for the spines, tough leaves, and difficult-to-eat growth forms found in many plants, a striking example of coevolution.

DEUTEROSTOMATE ANIMALS 595

Primates and the Origins of Humans

Another eutherian lineage that has had dramatic effects on ecosystems worldwide is the primate lineage, to which humans belong. Primates have undergone extensive recent evolutionary radiation. They probably descended from small arboreal (tree-living) insectivores sometime during the Cretaceous period. The major traits that distinguish primates from other mammals are all adaptations to arboreal life. They include:

- ▶ Dexterous hands with opposable thumbs that can grasp branches and manipulate food.
- ▶ Nails rather than claws.
- ▶ Eyes on the front of the face that provide good depth perception.
- ▶ Very small litters of offspring (usually just one) that receive extended parental care.

Early in its evolutionary history, the primate lineage split into two main branches, prosimians and anthropoids (Figure 33.25). Prosimians — lemurs, bush babies, and lorises—once lived on all continents, but today they are restricted to Africa, Madagascar, and tropical Asia. All of the mainland species are arboreal and nocturnal (Figure 33.26). However, on Madagascar, the site of a remarkable prosimian radiation, there are also diurnal and terrestrial species.

The anthropoids —tarsiers, monkeys, apes, and humans—evolved from an early primate lineage about



(a) *Propithecus verreauxi*

(b) *Galago senegalensis*

33.26 Prosimians

(a) The sifaka lemur is one of the many lemur species of Madagascar, where they are part of a unique assemblage of plants and animals, (b) The lesser bush baby is common in savannas over much of Africa. Its large eyes tell us that it is nocturnal.

596 CHAPTER THIRTY-THREE



(a) *Leontopithecus rosalia* (b) *Macaca sylvanus*

33.27 Monkeys

(a) Golden lion tamarins, are endangered New World monkeys living in coastal Brazilian rainforests. (b) Many Old World species, such as these Barbary macaques, live in social groups. Here two members of a group groom each other.

55 million years ago in Africa or Asia. New World monkeys have been evolving separately from Old World monkeys long

enough that they could have reached South America from Africa when those two continents were still close to each other. Perhaps because tropical America has been

heavily forested for a long time, all New World monkeys are arboreal (Figure 33.27a). Many of them have long, prehensile tails with which they can grasp branches. Many Old World primates are arboreal as well, but a number of species are terrestrial. Some of these species, such as baboons and macaques, live and travel in large groups (Figure 33.27b). No Old World primates have prehensile tails.

About 20 mya, the lineage that leads to modern



(a) *Hylobates lar*

(c) *Pongo pygmaeus* (b) *Pan paniscus*

(d) *Gorilla gorilla* 33.28 Apes

(a) Gibbons are the smallest of the apes. This common gibbon is found in Asia, from India to Borneo. (b) Chimpanzees, our closest relatives, are found in forested regions of Africa, (c) Orangutans live in the forests of Indonesia, (d) Gorillas, the largest apes, are restricted to humid African forests. This male is a lowland gorilla.

DEUTEROSTOMATE ANIMALS 597

Dots indicate the sites where australopithecine fossils have been found.

Adult female australopith

apes separated from the other Old World primates. The first apes were arboreal, but some species came to live in drier habitats with scattered trees, where they obtained most of their food on the ground. Apes are known to have lived in Africa, the Near East, and Asia 15-20 mya. Africa was especially rich in ape species, but the DNA sequences of living primates and some fossil evidence suggests that a European ape that dispersed into Africa about 10 mya may be the ancestor of modern apes. Four of the living genera of apes—gorillas (*Gorilla*), chimpanzees (*Pan*), orangutans (*Pongo*), and gibbons (*Hylobates*) — are restricted to tropical Africa and Asia (Figure 33.28). The fifth (*Homo*) has a worldwide distribution.

Human ancestors descended to the ground

The primate lineage that led to humans began with the ardipithecines. These apes had distinct morphological adaptations for bipedalism — locomotion in which the body is held erect and moved exclusively by movements of the hind legs. Bipedal locomotion frees the hands to manipulate objects and to carry them while walking. It also elevates the eyes, enabling the animal to see over tall vegetation to spot predators and prey. Both advantages were probably important for early ardipithecines and their descendants, the australopithecines.

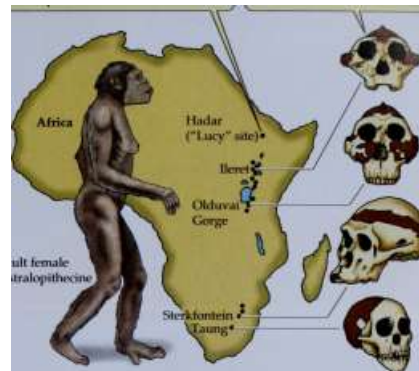
The first australopithecine skull was found in South Africa in 1924; since then other fragments have been found in a number of sites in Africa (Figure 33.29). The most complete fossil skeleton of an australopithecine, approximately 3.5 million years old, was discovered in Ethiopia in 1974. That individual, a young female known to the world as Lucy, attracted a great deal of attention because her remains were so complete and well preserved. Lucy has been assigned to the species *Australopithecus afarensis*, the most likely ancestor of humans. All the evidence from different parts of her skeleton suggests that Lucy was only about 1 meter tall and walked upright.

From *Australopithecus afarensis* ancestors, a number of species of australopithecines evolved. Several million years ago, two distinct types of australopithecines lived together over much of eastern Africa. The larger type (about 40 kg) is represented by at least two species, both of which died out suddenly about 1.5 million years ago. The 25-30-kg *A. africanus* is much rarer as a fossil, suggesting that it was less common than the other species.

Humans arose from australopithecine ancestors

Many experts believe that the recently discovered *Australopithecus garhi* or a similar species gave rise to the genus *Homo*. *A. garhi*, a small-brained, big-toothed hominid with

Lighter color indicates bones found at excavations; darker areas were reconstructed with modern materials.



Ileret, Kenya

Olduvai Gorge, Tanzania *Australopithecus robustus*

Sterkfontein, South Africa *Australopithecus africanus*

Taung, South Africa The first australopithecine skull found, in 1924, was a young individual

33.29 Australopithecine Fossils

Few fossilized remains are complete, but skull shapes can be reconstructed accurately.

humanlike leg proportions, began using tools to obtain food about 2.5 mya. They butchered animals to get at bone marrow, which is rich in fat.

Early hominids —members of the genus *Homo*—lived contemporaneously with australopithecines for perhaps half a million years. Two major changes accompanied the evolution of *Homo* from *Australopithecus*: an increase in body size and a doubling of brain size.

The oldest fossil remains of a member of the genus *Homo*, named *H. habilis*, were discovered in the Olduvai Gorge, Tanzania, and are estimated to be 2 million years old. Other fossils of *H. habilis* have been found in Kenya and Ethiopia. Tools used by these early hominids were found with the fossils. *H. habilis* lived in relatively dry areas where, for much of the year, the main food reserves are subterranean roots, bulbs, and tubers. To exploit these food resources, an animal must dig into hard, dry soils, something that cannot be done with an unaided primate hand. However, roots can be dug in large quantities in a relatively short time by an individual with a simple digging tool. *H. habilis* females carrying infants could have done so, freeing males to hunt animal prey to provide the proteins that roots lack.

The only other known extinct species of our genus, *Homo erectus*, evolved in Africa about 1.6 million years ago. Soon thereafter it had spread as far as eastern Asia. As it expanded its range and increased in abundance, *H. erectus* may have exterminated *H. habilis*. Members of *H. erectus* were as large as modern people, but their bones were considerably heavier. *H. erectus* used fire for cooking and for hunting large animals, and made characteristic stone tools

598 CHAPTER THIRTY-THREE

33.30 A Probable Human Phylogeny

The evolution of *Homo* from *Australopithecus* was marked by an increase in body size and a doubling of brain size.

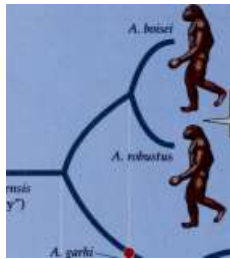
Ardipithecine

ancestor

(bipedalism)

A. boisei

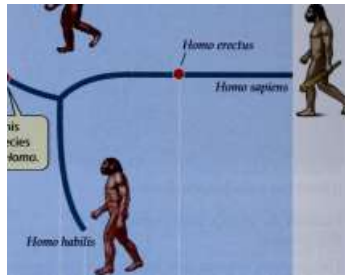
Australopithecus *A. afarensis* *A. anamensis* ("Lucy")



Australopithecine lineages probably coexisted with Homo for about 500,000 years.

A. garhi

Many experts believe this recently discovered species gave rise to the genus Homo.



Homo habilis

5.0

4.0

3.0 2.0

Millions of years ago (mya)

1.0

Present

that have been found in many parts of the Old World. These tools were probably used for digging, capturing animals, cleaning and cutting meat, scraping hides, and cutting wood. Although *H. erectus* survived in Eurasia until about 250,000 years ago, it was replaced in tropical regions by our species, *Homo sapiens*, about 200,000 years ago.

Brains steadily became larger

The trends that accompanied the transition from *Australopithecus* to *H. erectus* continued during the evolution of our own species (Figure 33.30). The earliest members of *Homo sapiens* had larger brains than members of the earlier species of *Homo*, a change that was probably favored by an increasingly complex social life. The ability of group members to communicate with one another was valuable for cooperative hunting and gathering and for improving one's status in the complex social interactions that must have characterized those societies, just as they do ours today.

Several types of *H. sapiens* existed during the mid-Pleistocene epoch, from about 1.5 million to about 300,000 years ago. All were skilled hunters of large mammals, but plants continued to be important components of their diets. During this period another distinctly human trait emerged: rituals and a concept of life after death. Deceased individuals were buried with tools and clothing, presumably for their existence in the next world.

One type of *H. sapiens*, generally known as Neanderthals because they were first discovered in the Neander Valley in Germany, was widespread in Europe and Asia between

about 75,000 and 30,000 years ago. Neanderthals were short, stocky, and powerfully built humans whose massive skulls housed brains somewhat larger than our own. They manufactured a variety of tools and hunted large mammals, which they probably ambushed and subdued in close combat. For a short time, their range overlapped that of a more modern form of *H. sapiens* known as Cro-Magnons, but then the Neanderthals abruptly disappeared. Many scientists believe that they were exterminated by the Cro-Magnons, just as *H. habilis* may have been exterminated by *H. erectus*.

Cro-Magnon people made and used a variety of sophisticated tools. They created the remarkable paintings of large mammals, many of them showing scenes of hunting, that have been discovered in caves in various parts of Europe (Figure 33.31f). The animals depicted were characteristic of the cold steppes and grasslands that occupied much of Europe during periods of glacial expansion. Cro-Magnon people spread across Asia, reaching North America perhaps as early as 20,000 years ago, although the date of their arrival in the New World is still uncertain. Within a few thousand years they had spread southward through North America to the southern tip of South America.

Humans evolved language and culture

As our ancestors evolved larger brains, their behavioral capabilities increased, especially the capacity for language. Most animal communication consists of a limited number of signals, which pertain mostly to immediate circumstances

DEUTEROSTOMATE ANIMALS 599

(«)

33.3 7 Hunting, Pastoralism, and Agriculture

(a) Cro-Magnon cave drawings such as those found in Lascaux Cave in France typically depict the large mammals that they hunted, (b) The Masai are a pastoral people living on East African savannas, where they and their cattle typically coexist with native grazing and browsing mammals, (c) Intense agricultural development totally transforms the landscape. These rice terraces are on the island of Bali in Indonesia.

and axe associated with changed emotional states induced by those circumstances. Human language is far richer in its symbolic character than any other animal vocalizations. Our words can refer to past and future times and to distant places. We are capable of learning thousands of words, many of them referring to abstract concepts. We can rearrange words to form sentences with complex meanings.

The expanded mental abilities of humans are largely responsible for the development of culture, the process by which knowledge and traditions are passed along from one generation to another by teaching and observation. Culture can change rapidly because genetic changes are not necessary for a cultural trait to spread through a population. The primary disadvantage of culture is that its norms must be taught to each generation. The tools and other implements associated with human fossils, as well as the cave paintings early humans created, reveal cultural traditions.

Cultural learning greatly facilitated the spread of domesticated plants and animals and the resultant conversion of most human societies from ones in which food was obtained by hunting and gathering to ones in which pastoralism (herding large animals) and agriculture dominated (Figure 33.31b,c). The development of agriculture led to an increasingly sedentary life, the growth of cities, greatly expanded food supplies, a rapid increase in the human population, and the appearance of occupational specializations, such as artisans and healers.

Agriculture developed in the Middle East approximately 11,000 years ago. From there it spread rapidly northwest-



(b)



'ft** Ai&

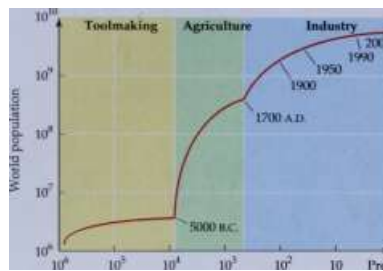
(c)

ward across Europe, finally reaching the British Isles about 4,000 years ago. The first plants and animals to be domesticated were cereal grains such as wheat and barley; legumes (beans, lentils, and peas); and woody plant crops such as grapes and olives. Others, such as rye, cabbage, celery, and carrots, were domesticated later. Cattle, sheep, goats, horses, dogs, and cats were the most important domesticated animals.

Agriculture developed independently in eastern Asia, contributing to our modern diet soybeans, rice, citrus fruits,

Industry

^2000 1990 1950 1900



10' 10 J 1(T

Years before present

Present

33.32 Human Population Surges

The human population surged following (1) the invention of tools, (2) the domestication of plants and animals, and (3) the Industrial Revolution.

mangoes, pigs, and chickens. There was some exchange, even at early times, among agricultural centers in the Old World, but when people spread across the cold and barren Bering land bridge into the New World, they apparently brought no domesticated plants with them. These people subsequently developed rich and varied agricultural systems based on corn, tomatoes, kidney and lima beans, peanuts, potatoes, chili peppers, and squashes. The largest animals domesticated by humans in the New World were llamas and alpacas in the Andes of South America—animals not large enough to carry a person. The Amerindians of Mexico and Central America had no domesticated animals larger than turkeys.

The human population has grown rapidly

The human population has experienced three major phases of increase (Figure 33.32). The first, stimulated by tool use, lasted about a million years. At the end of that period, the human population is estimated to have been approximately 5 million. During the second surge, which followed the domestication of plants and animals and the invention of agriculture, the human population may have increased to about 500 million people within 8,000 years.

We are currently in the middle of the third great population surge, triggered in the eighteenth century by the Industrial Revolution. In the industrialized countries of the world, death rates fell and life spans increased. By the end of the nineteenth century, human numbers had passed 1 billion. Despite the devastation of two World Wars and countless minor conflicts, the twentieth century saw the human population reach its current level of more than 6 billion. It is projected to increase to more than 11 billion by 2050.

The first two human population surges were followed by periods of relative stability. Whether the current surge will follow the same pattern, at what size it might level off, what hardships might ensue if it does not, and what the

consequences will be for other species are questions that are fiercely debated. We will discuss these issues further in Chapter 58.

irn



Chapter Summary

Deuterostomes and Protostomes

- The deuterostome lineage separated from the protostome lineage early in the history of animal life on Earth.
- There are fewer major lineages and fewer species of deuterostomes than protostomes, but as members of the deuterostome lineage we have a special interest in its members. Review Figure 33.1

Echinoderms: Complex Biradial Symmetry

- Echinoderms have a radially symmetrical body plan, a unique water vascular system, and a calcified internal skeleton. Review Figure 33.2a
- Nearly all living species of echinoderms have a bilaterally symmetrical, ciliated larva that feeds as a planktonic organism. Review Figure 33.2b

- ▶ Six major groups of echinoderms survive today, but 23 other lineages existed in the past. Some groups of echinoderms have arms, but others do not.

Chordates: New Ways of Feeding

- ▶ Evolution among the hemichordates and chordates led to new ways of capturing and handling food.
- ▶ The large proboscis of acorn worms is both a digging and a food-capturing organ.
- ▶ Members of the chordates evolved enlarged pharyngeal slits as feeding devices and a dorsal supporting rod, the notochord.
- ▶ Tunicates are sessile as adults and filter prey from seawater with large pharyngeal baskets. Their larvae have notochords and dorsal, hollow nerve cords.

Origin of the Vertebrates

- ▶ Vertebrates evolved jointed internal skeletons centered around a vertebral column, a body plan that enabled them to swim rapidly. Early vertebrates fed by filtering small animals from mud. Review Figure 33.7
- ▶ Jaws evolved from anterior gill arches and enabled their possessors to grasp and chew their prey, expanding food sources and improving nutrition. Jawed fishes rapidly became the dominant animals in both marine and fresh waters. Review Figures 33.8, 33.10
- ▶ Fishes evolved unjointed fins with which they could control their swirring movements and stabilize themselves in the water, and lunglike sacs that helped them stay suspended in open water.
- ▶ Bony fishes come in a wide variety of sizes and shapes, and many species have complex behaviors.

Colonizing the Land: Obtaining Oxygen from the Air

- ▶ Two fish lineages—lobe-fins and lungfishes—evolved jointed fins. Amphibians, the first terrestrial vertebrates, arose from one of these lineages. Review Figure 33.14
- ▶ Most amphibians live in water at some time in their lives, and their eggs must remain moist. Review Figure 33.16
- ▶ About 4,500 species of amphibians live today. They belong to three orders: caecilians, frogs and toads, and salamanders.

DEUTEROSTOMATE ANIMALS 601

- ▶ Amniotes evolved eggs with shells impermeable to water and thus became the first vertebrates to be independent of water for breeding. Review Figure 33.17
- ▶ Modern reptiles are members of four lineages—turtles and tortoises; tuataras; snakes and lizards; and crocodilians. Review Figure 33.18
- ▶ Dinosaurs rose to dominance about 215 mya and dominated terrestrial environments for about 150 million years until their extinction at the end of the Mesozoic era.

Birds: Feathers and Flight

- ▶ Birds arose about 175 mya, but much controversy surrounds their origins.
- ▶ The 9,600 species of living birds are characterized by feathers, high metabolic rates, and parental care.

The Origin and Diversity of Mammals

- ▶ Mammals evolved during the Mesozoic era, about 225 mya.
- ▶ Eggs of mammals are fertilized within the bodies of females, and embryos develop there for some time before being born. Mammals are unique in suckling their young with milk secreted by mammary glands.
- ▶ The three species of mammals in subclass Prototheria lay eggs, but all other mammals give birth to developed young.
- ▶ Therian mammals are divided into two major groups: the marsupials, which give birth to tiny young that are raised in a pouch on the female's belly, and eutherians, which give birth to relatively well-developed offspring.

Primates and the Origins of Humans

- ▶ The primates split into two major lineages, one leading to the prosimians—lemurs, lorises, and pottos—and the other leading to the anthropoids—tarsiers, monkeys, apes, and humans. Review Figure 33.25
- ▶ Hominids evolved in Africa from terrestrial, bipedal ancestors. Review Figures 33.29, 33.30
- ▶ Early humans evolved large brains, language, and culture. They made and used tools, developed rituals, and domesticated plants and animals. In combination, these traits enabled humans to increase greatly in numbers.

► The human population has increased greatly three times. We are currently in the middle of the third population surge. When and how it will end is hotly debated. Review Figure 33.32

For Discussion

1. In what animal phyla has the ability to fly evolved? How do structures used for flying differ among these animals?
2. Extracting suspended food from the water column is a common mode of foraging among animals. Which groups contain species that extract prey from the air? Why is this mode of obtaining food so much less common than extracting prey from water?
3. Large size both confers benefits and poses certain risks. What are these risks and benefits?
4. Amphibians have survived and prospered for many millions of years, but today many species are disappearing and populations of others are declining seriously. What features of amphibian life histories might make them especially vulnerable to the kinds of environmental changes now happening on Earth?
5. The evolution of jaws allowed vertebrates to utilize a remarkably wide array of food types; yet jawless animals are able to eat most of those kinds of foods. Compare the ways that jawed and jawless animals would eat the following kinds of food:
 - a. a snail
 - b. an insect
 - c. a fish
 - d. a bird
 - e. a plant leaf
6. The body plan of all vertebrates is based on four appendages. Describe the varied forms that these appendages take and how they are used. How do the vertebrates that have lost their four appendages move?
7. Compare the ways that different animal lineages colonized the land. How were those ways influenced by the body plans of animals in the different lineages?

Part Five

The Biology of

Flowering Plants



34

The Plant Body



\[m The oldest known plant is a bristlycone

pine that has been living for more than 4,900
years —almost 50 centuries. In contrast, it is
"hardly doubtful that any animal has ever lived as long
as 2 centuries. The extreme ages achieved by
some trees prove that plants can cope very successfully
with their environments.

Plants cannot move, but they have mechanisms for coping with environmental changes that they can't escape. They create and maintain an internal environment that differs from the external environment. They also regulate their own metabolism, which enables them to perform their necessary functions.

Motion is not a characteristic of plants; instead, we may think of plants as "growing machines." By growing, plants accomplish some of the same things that animals achieve through motion. Growing roots, for example, can reach into new supplies of water and nutrients.

Although they have simpler nutritional needs than animals do, plants must nevertheless obtain nutrients—not only the raw materials of photosynthesis (carbon dioxide and water), but also mineral elements such as nitrogen, potassium, and calcium. Seed plants—even the tallest trees—transport water from the soil to their tops, and they transport the products of photosynthesis from the leaves to their roots and other parts.

Plants also interact with their living and nonliving environments. They respond to environmental cues as they grow and develop. Their responses are mediated by chemical signals that move within cells and throughout the plant body. Among the resulting changes are ones that lead to growth, development, and reproduction.

Because we can understand the function of these growing machines only in terms of their underlying structure, this chapter focuses on the structure of the plant body, with primary emphasis on flowering plants. We'll examine plant structure at the level of

An Ancient Individual

Bristlecone pines (*Pinus aristata*) can live for centuries. The oldest known living organism is a bristlecone pine that has been alive for almost 5,000 years—long enough to have witnessed all of recorded human history.

the organs, cells, tissues, and tissue systems. Then we'll see how meristems—organized groups of dividing cells—serve the growth of the plant body, both in length and, in woody plants, in width. The chapter concludes with a consideration of how leaf structure supports photosynthesis.

Vegetative Organs of the Flowering Plant Body

You will recall from Chapter 29 that flowering plants (angiosperms) are tracheophytes that are characterized by double fertilization, a triploid endosperm, and seeds en-



The possession of a single cotyledon clearly distinguishes the monocots from the other angiosperms. Several other anatomical characteristics also differ between the monocots and the eudicots. Most angiosperms that do not belong to either lineage resemble eudicots in the characteristics shown here.

Cotyledons

Veins in leaves

Arrangement of

primary vascular

Flower parts bundles in stem

Monocots



One

Two

closed in modified leaves called Eudicots carpels. Their xylem contains cells called vessel elements and fibers, and their phloem contains companion cells.

Flowering plants possess three kinds of vegetative (nonreproduc-

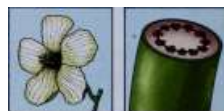
tive) organs: roots, stems, and leaves. Flowers, which are the plant's devices for sexual reproduction, consist of other types of organs that will be considered in a later chapter.

Most flowering plants belong to one of two major lineages. Monocots (Monocotyledones) are generally narrow-leaved flowering plants such as grasses, lilies, orchids, and palms. Eudicots (Eudicotyledones) are broad-leaved flowering plants such as soybeans, roses, sunflowers, and maples. These two monophyletic classes account for 97 percent of the species of flowering plants (Figure 34.1). Most of the remaining species (including water lilies and the lineage that includes magnolias) are structurally similar to the eudicots.*



Usually parallel Usually in Scattered

multiples of three



Usually netlike

Usually in fours or fives

In a ring

The basic body plans of a generalized monocot and a generalized eudicot are shown in Figure 34.2. In both lineages, the vegetative plant body consists of two systems: the shoot system and the root system.

The shoot system of a plant consists of the stems and the leaves, as well as flowers (which contain leaflike parts). Broadly speaking, the leaves are the chief organs of photosynthesis. The stems hold and display the leaves to the sun and provide connections for the transport of materials between roots and leaves. The points where leaves attach to a stem are called nodes, and the stem regions between successive nodes are internodes.

traditionally, botanists have referred to all flowering plants other than monocots as dicots. However, the dicots do not constitute a monophyletic lineage (see Figure 29.13). Because we wish to emphasize lineages, we do not use that term here.

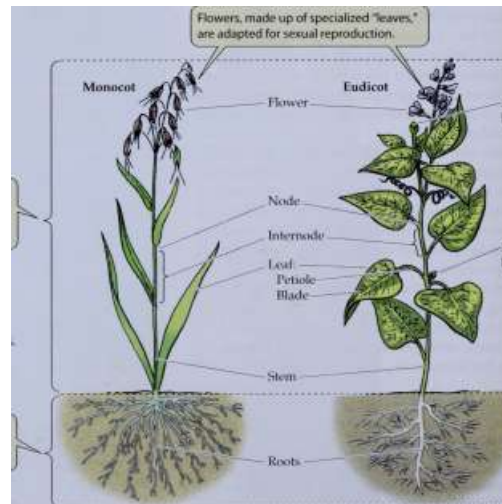
34.2 Vegetative Organs and Systems

The basic plant body plan and the principal vegetative organs are similar in monocots and eudicots.

The root system anchors and provides nutrients for a shoot system.

Flowers, made up of specialized "leaves," are adapted for sexual reproduction.

The shoot system consists of stems and leaves, in which photosynthesis takes place.



Apical bud

Lateral bud

(a)



34.3 Root Systems

The taproot system of a beet (a) contrasts with the fibrous root system of a grass (b)

The root system provides support and nutrition. Roots anchor the plant in place, and their extreme branching and high surface area-to-volume ratio adapt them to absorb water and mineral nutrients from the soil.

Each of the vegetative organs can be understood in terms of its structure. By structure we mean both its overall form and the anatomy of its component cells and tissues, as well as their arrangement. Let's first consider the overall forms of roots, stems, and leaves.

Roots anchor the plant and take up water and minerals

Water and minerals usually enter the plant through the root system, which usually lies in the soil, mostly in complete darkness. There are two principal types of root systems. Many eudicots have a taproot system: a single, large, deep-growing primary root accompanied by less prominent lateral roots (Figure 34.3a). The taproot itself often functions as a food storage organ, as in carrots and radishes.

By contrast, monocots and some eudicots have a fibrous root system, which is composed of numerous thin roots roughly equal in diameter (Figure 34.3b). Many fibrous root systems have a large surface area for the absorption of water and minerals. A fibrous root system holds soil very well. Grasses with fibrous root systems, for example, may protect steep hillsides where runoff from rain would otherwise cause erosion.

Some plants have what are called adventitious roots. These roots arise from points along the stem where roots would not usually occur; some even arise from the leaves. In many species, adventitious roots also form when a piece of shoot is cut from the plant and placed in water or soil. Adventitious rooting enables the cutting to establish itself in the soil. Some plants—corn, banyan trees, and some palms, for example—use adventitious roots as props to help support the shoot.

THE PLANT BODY 605

Stems bear buds, leaves, and flowers

Unlike roots, stems bear buds of various types. A bud is an embryonic shoot. A stem bears leaves at its nodes, and where each leaf meets the stem there is a lateral bud (see Figure 34.2), which can develop into a new branch, or extension of the stem, if it becomes active. The branching patterns of plants are highly variable, depending on the species, environmental conditions, and a gardener's pruning activities.

At the tip of each stem or branch is an apical bud, which produces the cells for the growth and development of that stem or branch. At times that vary depending on the species, buds form that develop into flowers. Some stems are highly modified. The tuber of a potato, for example—the part of the plant eaten by humans—is a portion of the stem rather than a root. Its "eyes" contain lateral buds; thus, a sprouting potato is just a branching stem (Figure 34.4rt). The runners of strawberry plants and Bermuda grass are horizontal stems from which roots grow at frequent intervals (Figure 34.4b). If the links between the rooted portions are broken, independent plants can develop on each side of the break. This is a form of vegetative reproduction, which we will discuss in a later chapter.

Unlike most roots, stems may be green and capable of photosynthesis. But stems are not the principal sites of photosynthesis.

Leaves are the primary sites of photosynthesis

In gymnosperms and most flowering plants, the leaves are responsible for most of the plant's photosynthesis, producing energy-rich organic molecules and releasing oxygen gas. Most leaves also carry out metabolic reactions that make nitrogen available to the plant for the synthesis of proteins and nucleic acids. In certain plants, leaves are highly modified for more specialized functions, as we will see below.

As photosynthetic organs, leaves are marvelously adapted for gathering light. Typically, the blade of a leaf is a thin, flat structure attached to the stem by a stalk called a petiole. During the daytime the leaf blade is held by its petiole at an angle almost perpendicular to the rays of the sun. This placement, with the leaf surface facing the sun, maximizes the amount of light available for photosynthesis. Some leaves track the sun, moving so that they constantly face it. If leaves were thicker than they are, the outer layers of cells would absorb so much of the light that the interior layers would be too dark and would be unable to photosynthesize.

The leaves at different sites on a single plant may have quite different shapes. These shapes result from a combination of genetic, environmental, and developmental influences. Most species, however, bear leaves of a particular broadly defined type. A leaf may be simple, consisting of a



34.4 Modified Stems

(a) A potato is a modified stem called a tuber; the sprouts that grow from its eyes are branches, not roots, (b) Runners produce roots at intervals, providing a local water supply and allowing rooted portions to live independently if the runner is cut. (c) The thorny spines are leaves on the enlarged stem of a cactus plant.



The runners of beach strawberry are horizontal stems.

Root

single blade, or compound, with blades, or leaflets, arranged along an axis or radiating from a central point (Figure 34.5). In a simple leaf, or in a leaflet of a compound leaf, the veins may be parallel to one another, as in mono-cots, or in a netlike arrangement, as in eudicots.

The general development of a specific leaf pattern is programmed in the plant's genes and is expressed by differential growth of the leaf veins and of the tissue between the veins. As a result, plant taxonomists have often found leaf forms (outlines, margins, tips, bases, and patterns of arrangement) to be reliable characters for classification and identification. At least some of the forms in Figure 34.5 probably look familiar to you.

In some plant species, leaves are highly modified for special functions. Some leaves serve as storage depots for energy-rich molecules, as in the bulbs of onions. In other species—some of the succulents—the leaves store water. The spines of cacti are modified leaves. Certain leaves of poinsettias, dogwoods, and some other plants are brightly colored and help attract pollinating animals to the often less striking flowers. Many plants, such as peas, have tendrils—modified leaves that support the plant by wrapping around other plants.

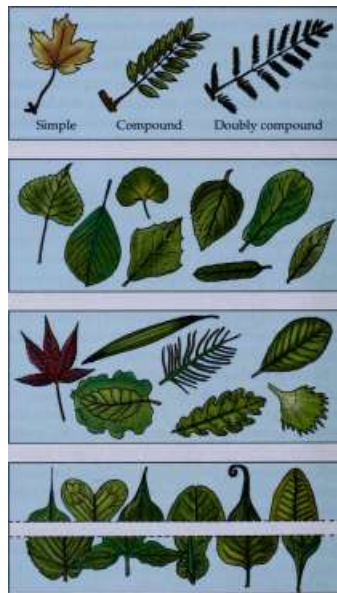
Leaves and other plant organs are composed of cells, tissues, and tissue systems. Let's now consider plant cells— the basic building blocks of plant organs.

Shapes

Margins

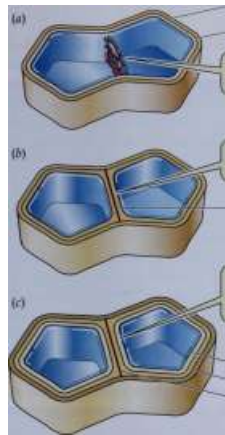
Apices

Bases



34.5 The Diversity of Leaves

Simple leaves are those with a single blade. Compound leaves consist of leaflets arranged along a central axis. Further division of leaflets results in a doubly compound leaf. Other characters of leaves can also be used to identify plants.



Middle lamella Primary cell wall

The cell plate is the first barrier to form.

Each daughter cell secretes a primary wall.

Middle lamella

After the cell stops expanding, it may secrete more layers forming secondary walls.

Secondary wall Primary wall Middle lamella

34.6 Cell Wall Formation

Cell walls form as the final step in plant cell division.

Plant Cells

Living plant cells have all the essential organelles common to eukaryotes (see Figure 4.7). In addition, they have some distinguishing structures and organelles.

- Some plant cells contain chloroplasts or other plastids.
- Many plant cells contain vacuoles.
- Every plant cell is surrounded by a cellulose-containing cell wall.

Although most kinds of plant cells are alive when they perform their functions, certain others function only after their

THE PLANT BODY 607

living parts have died and disintegrated. Other plant cells develop special chemical capabilities; for example, some can

perform photosynthesis, and others produce and secrete waterproofing materials. Several plant cell types differ dramatically in the structure of their cell walls.

Cell walls may be complex in structure

The cytokinesis of a plant cell is completed when cell walls form, separating the two daughter cells. The daughter cells secrete a glue that constitutes the middle lamella, which forms a layer between them. Then each daughter cell secretes cellulose and other polysaccharides to form a primary wall, which continues to grow as the cell grows to its final size (Figure 34.6).

Once cell expansion stops, a plant cell may deposit more polysaccharides, sometimes impregnated with further materials—such as lignin, characteristic of wood, or suberin, a complex lipid characteristic of cork—in one or more layers internal to the primary wall. These layers collectively form the secondary wall, which often serves supporting or waterproofing roles.

Although the cell wall lies outside the plasma membrane of the cell, it is not a chemically inactive region. Chemical reactions in the wall play important roles in cell expansion and defense. Cell walls may thicken or be sculpted or perforated as part of differentiation into specialized cell types. Except where the secondary wall is made waterproof by added substances, the wall is porous to water and to most small molecules.

Localized modifications in the walls of adjacent cells allow water and dissolved materials to move easily from cell to cell. In cells that have not developed a secondary wall, the primary wall usually has thin regions. Strands of cytoplasm called plasmodesmata (singular plasmodesma) pass through the primary wall in these regions, allowing substances to move freely from cell to cell without having to cross a plasma membrane (Figure 34.7a). The plasmodes-

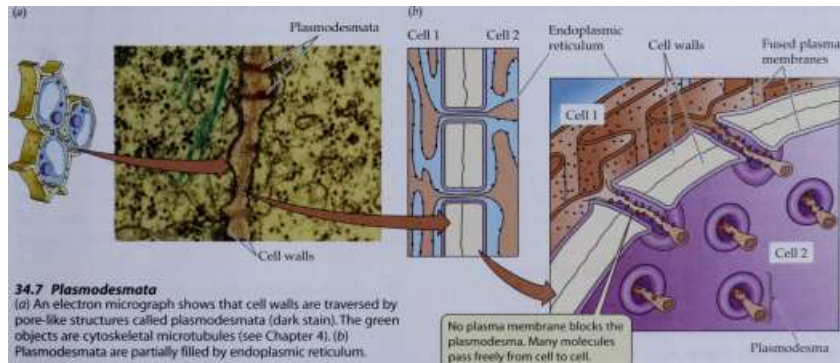
34.7 Plasmodesmata

(a) An electron micrograph shows that cell walls are traversed by pore-like structures called plasmodesmata (dark stain).The green objects are cytoskeletal microtubules (see Chapter 4). (b) Plasmodesmata are partially filled by endoplasmic reticulum.

Endoplasmic

reticulum Cell walls

Fused plasma membranes



No plasma membrane blocks the plasmodesma. Many molecules pass freely from cell to cell.

Plasmodesma

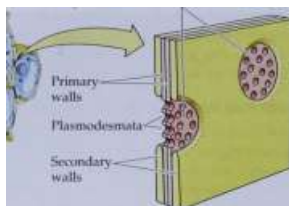
608 CHAPTER THIRTY-FOUR

Pits



-^.' Primary

1 walls



Plasmodesmata^—?jkO o]

Secondary walls

34.8 Pits Allow Materials to Move between Cells with Secondary Walls

Secondary cell walls may be interrupted by pits that allow the passage of water and other materials between cells.

Plasmodesmata allow direct communication between plant cells, as discussed in Chapter 15.

A plasmodesma is wide enough so that portions of the endoplasmic reticulum extend between cells (Figure 34.7b). Under certain circumstances, a plasmodesma can enlarge dramatically, allowing even macromolecules and viruses to pass directly between cells. Even in cells with a waterproofed secondary wall, water and dissolved materials can pass from cell to cell by way of structures called pits (Figure 34.8). Pits are interruptions in the secondary wall that leave the thin regions of the primary wall, and thus the plasmodesmata, unobstructed.

Parenchyma cells are alive when they perform their functions

The most numerous cell type in young plants is the parenchyma cell (Figure 34.9f). Parenchyma cells usually have thin walls, consisting only of a primary wall and the shared middle lamella. Many parenchyma cells have shapes similar to those of soap bubbles crowded into a limited space—shapes with 14 faces. They are usually not elongated or otherwise asymmetrical. Most have large central vacuoles.

The photosynthetic cells in leaves are parenchyma cells filled with chloroplasts. Some nonphotosynthetic parenchyma cells store substances such as starch or lipids. In the cytoplasm of these cells, starch is often stored in specialized plastids called leucoplasts. Lipids may be stored as oil droplets, also in the cytoplasm. Some parenchyma cells appear to serve as "packing material" and play a vital role in supporting the stem. Others retain the capacity to divide and hence may give rise to new cells, as when a wound results in cell proliferation.

Collenchyma cells provide flexible support while alive

Collenchyma cells are supporting cells that lay down pri-

cell walls that are characteristically thick in the cor-

of the cells (Figure 34.9b). Collenchyma cells are gener-

ally elongated. In these cells the primary wall thickens, but no secondary wall forms. Collenchyma provides support to leaf petioles, nonwoody stems, and growing organs. Tissue made of collenchyma cells is flexible, permitting stems and petioles to sway in the wind without snapping. The familiar "strings" in celery consist primarily of collenchyma.

Sclerenchyma cells provide rigid support after they die

In contrast to collenchyma, sclerenchyma cells have a thickened secondary wall that performs their major function: support. Many sclerenchyma cells function when dead. There are two types of sclerenchyma cells: elongated fibers and variously shaped sclereids. Fibers, often organized into bundles, provide relatively rigid support both in wood and in other parts of the plant (Figure 34.9c). The bark of trees owes much of its mechanical strength to long fibers. Sclereids may pack together densely, as in a nut's shell or in some seed coats (Figure 34.9d). Isolated clumps of sclereids, called stone cells, in pears and some other fruits give them their characteristic gritty texture.

Xylem transports water from roots to stems and leaves

The xylem of tracheophytes conducts water from roots to aboveground plant parts. It contains conducting cells called tracheary elements, which undergo programmed cell death before they assume their function of transporting water and dissolved minerals. The evolutionary more ancient tracheary elements, found in gymnosperms and other tracheophytes, are tracheids—spindle-shaped cells interconnected by numerous pits in their cell walls (Figure 34.9e). When the cell contents—nucleus and cytoplasm—disintegrate upon cell death, water can move with little resistance from one tracheid to its neighbors by way of pits.

Flowering plants evolved a water-conducting system made up of vessels. The individual cells that form vessels, called vessel elements, also die and become empty before they can transport water. These cells secrete a waterproofing substance into their cell walls, then break down their end walls, and finally die and disintegrate. The result is a hollow tube through which water can flow freely. Vessel elements are generally larger in diameter than tracheids; they are laid down end-to-end, so that each vessel is a continuous hollow tube consisting of many vessel elements and providing an open pipeline for water conduction (Figure 34.9f). In the course of angiosperm evolution, vessel elements have become shorter, and their end walls have become less and less obliquely oriented and less obstructed (Figure 34.10). The xylem of many angiosperms also includes tracheids.

Phloem translocates carbohydrates and other nutrients

The transport cells of the phloem, unlike those of the xylem, are living cells. In flowering plants the characteristic

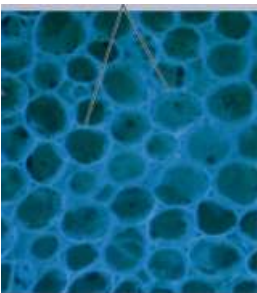
THE PLANT BODY 609

(a) Parenchyma cells

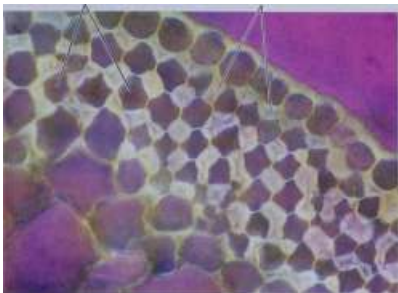
Cell walls

(/ ») Collenchyma cells

Cell walls



^ m r ~~~ . flBH ft Am



(d) Sclerenchyma: Sclereids Secondary cell walls

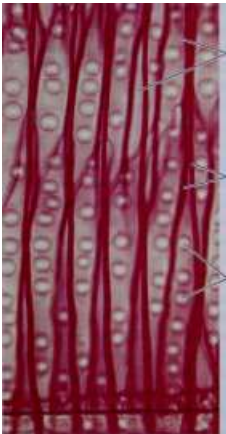


■

f •



o?)



Tracheids

Cell walls

Pits



Vessel I elements

EH=>- Secondary I cell wall

34.9 Plant Cells

(a) Parenchyma cells in the leaf of a primrose plant; note the uniform cell walls, (b) Collenchyma cells make up the five outer cell layers of this spinach leaf vein. They are recognizable because their cell walls are thick at the corners of the cells and thin elsewhere.

(c) Sclerenchyma: Fibers in a sunflower plant (*Helianthus*).The thickened walls are stained red.

(d) Sclerenchyma:These extremely thick secondary cell walls of sclereids are laid down in layers.They provide support and a hard texture to structures such as nuts and seeds, (e) Water-conducting tracheids in pine wood.The thick cell walls are stained dark red.

(o Vessel elements in the stem of a squash.The secondary walls are stained red; note the different patterns of thickening, including rings and spirals.

610 CHAPTER THIRTY-FOUR

Celll

\ esse!

\

Coll 2

Cell 3

:

^3



This type is the most recently evolved.

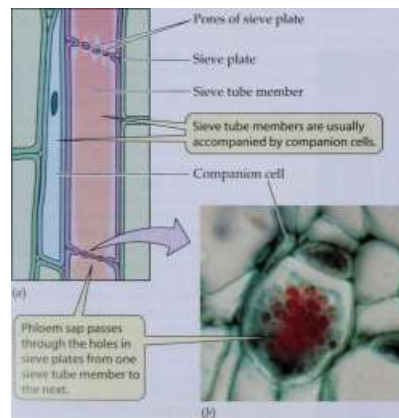


This cell type is evolutionarily the most ancient.

34.10 Evolution of the Conducting Cells of Vascular Systems

The xylem of tracheophytes has changed over time.The cells that conduct water and mineral nutrients have become shorter and the end walls have become more perpendicular to the side walls.

Pores of sieve plate Sieve plate



Phloem sap passes through the holes in sieve plates from one sieve tube member to the next.

34.11 Sieve Tubes

) Individual sieve tube members join to form long tubes that transport carbohydrates and other nutrient molecules, (b) Sieve plates form at the ends of each sieve tube member.

cell of the phloem is the sieve tube member (Figure 34.11a). Like vessel elements, these cells meet end-to-end. They form long sieve tubes, which transport carbohydrates and many other materials from their sources to tissues that consume or store them. In plants with mature leaves, for example, excess products of photosynthesis move from leaves to root tissues.

As sieve tube members mature, plasmodesmata in the end walls enlarge, enhancing the connection between the contents of neighboring cells. The result is end walls that look like sieves, and are called sieve plates (Figure 34.11b). As the holes in the sieve plates expand, the tonoplast (the membrane around the central vacuole) disappears. The nucleus and some of the other organelles in the sieve tube member also break down and thus do not clog the holes of the sieve.

At functional maturity, a sieve tube is filled with sieve tube sap, consisting of water, sugars, and other solutes. This mixture moves from cell to cell along the sieve tube, carrying its dissolved sugars and other important materials with it. At the periphery of a sieve tube member, next to the cell wall and distinct from the sieve tube sap, is a layer of cytoplasm. This stationary layer of cytoplasm confines the remaining organelles.

The sieve tube members have adjacent companion cells (see Figure 34.11a), produced along with the sieve tube member when a parent cell divides. Companion cells retain all their organelles and may, through the activities of their nuclei, regulate the performance of the sieve tube members.

All these kinds of plant cells play important roles. Next let's see how they are organized into tissues and tissue systems.

Plant Tissues and Tissue Systems

A tissue is an organized group of cells, working together as a functional unit. Parenchyma cells make up parenchyma tissue, a simple tissue—that is, a tissue composed of only one type of cell. Sclerenchyma and collenchyma are other simple tissues, composed, respectively, of sclerenchyma and collenchyma cells.

Different cell types also combine to form complex tissues. Xylem and phloem are complex tissues, composed of more than one type of cell. As a result of its cellular complexity, xylem can perform a variety of functions, including transport, support, and storage. The xylem of angiosperms contains vessel elements and tracheids as conducting cells, thick-walled sclerenchyma fibers for support, and parenchyma cells that store food. The phloem of angiosperms includes sieve tube members, companion cells, fibers, sclereids, and parenchyma cells.

Tissues, in turn, are grouped into tissue systems that extend throughout the body of the plant, from organ to organ. There are three tissue systems: vascular, dermal, and ground (Figure 34.12).

The vascular tissue system, which includes the xylem and phloem, is the conductive, or "plumbing," system of

The dermal tissue system is the outer covering of the plant.

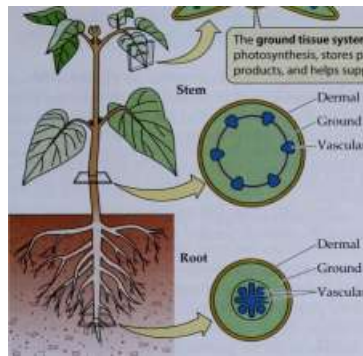
The vascular tissue system

conducts water and solutes throughout the plant.



The ground tissue system conducts photosynthesis, stores photosynthetic products, and helps support the plant.

Dermal /Of "XV/ Ground Vascular



34.12 Three Tissue Systems Extend throughout the Plant Body

The arrangement shown here is typical of eudicots.

the plant. All the living cells of the plant body require a source of energy and chemical building blocks. The phloem transports carbohydrates from sites of production—called sources (commonly the leaves)—to sites of utilization or storage—called sinks—elsewhere in the plant. The xylem distributes water and mineral ions taken up by the roots to the stem and leaves.

The dermal tissue system is the outer covering of the plant. All parts of the young plant body are covered by an epidermis, which may be a single layer of cells or several layers. The epidermis contains epidermal cells and may also include specialized cell types, such as the guard cells that form stomata (pores) in leaves. The shoot epidermis secretes a layer of wax-covered cutin, the cuticle, that helps retard water loss from stems and leaves. The stems and roots of woody plants have an additional protective covering called the periderm, which will be discussed later in this chapter.

The ground tissue system makes up the rest of a plant and consists primarily of parenchyma tissue, often supplemented by collenchyma or sclerenchyma. Ground tissue functions primarily in storage, support, photosynthesis, and the production of defensive and attractive substances.

In the discussions that follow, we'll examine how the tissue systems are organized in the different organs of a flowering plant. Let's begin by seeing how this organization develops as the plant grows.

Forming the Plant Body

In its early embryonic stages, a plant establishes the basic body plan for its mature form (Figure 34.13). Two patterns contribute to the plant body plan:

- The apical-basal pattern is the arrangement of cells and tissues along the main axis from root to shoot.
- The radial pattern is the concentric arrangement of tissue systems.

Both patterns arise through orderly development and are best understood in developmental terms.

Plants and animals develop differently

As the plant body grows, it may lose parts, and it forms new parts that may grow at different rates. The growing stem consists of modules or units, laid down one after another. Each unit consists of a node with its attached leaf or leaves, the internode below that node, and the lateral bud or buds at the base of that internode. New units are formed as long as the stem continues to grow.

Each branch of a plant may be thought of as a unit that is in some ways independent of the other branches. A branch of a plant does not bear the same relationship to the remainder of the plant body as an arm does to the remainder of the human body. Among other things, branches form one after another, unlike arms, which form simultaneously during embryonic development. Also, branches often differ from one another in number of leaves and in the degree to which they themselves branch.

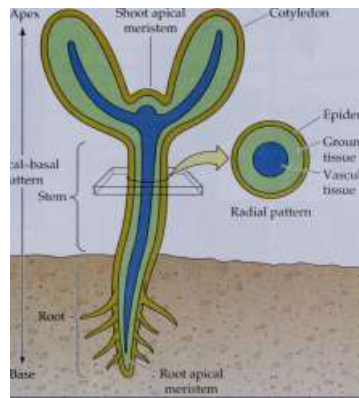
Cotyledon

Apical-basal pattern

Stem

Epidermis

Ground tissue Vascular tissue



ipi

meristem

34.13 Patterns in the Plant Body Plan

The embryonic plant establishes the basic body plan for its mature form. Two patterns are evident in this body plan: the apical-basal pattern and the radial pattern.

612 CHAPTER THIRTY-FOUR

Leaves are units of another sort, produced in fresh batches to take over the daily function of gathering energy for the plant. Leaves are usually short-lived, lasting weeks to a few years. Branches and stems are longer-lived, lasting from years to centuries.

Root systems are also branching structures, and lateral roots are semi-independent units. As the root system grows, penetrating and exploring the soil environment, many roots die and are replaced by new ones.

All parts of the animal body grow as an individual develops from embryo to adult, but this growth is determinate. That is, the growth of the individual and its parts ceases when the adult state is reached. The growth of stems and roots, by contrast, is indeterminate and is generated from specific regions of active cell division and cell expansion.

The localized regions of cell division in plants are called meristems. Meristematic tissues are forever young, retaining the ability to produce new cells indefinitely. They are comparable to the stem cells found in animals. When a meristem cell divides, the two resulting cells initially take up no more volume than did the single cell prior to division. One daughter cell develops into another meristem cell the size of its parent, while the other daughter cell develops into a more specialized cell.

A hierarchy of meristems generates a plant's body

There are two types of meristems:

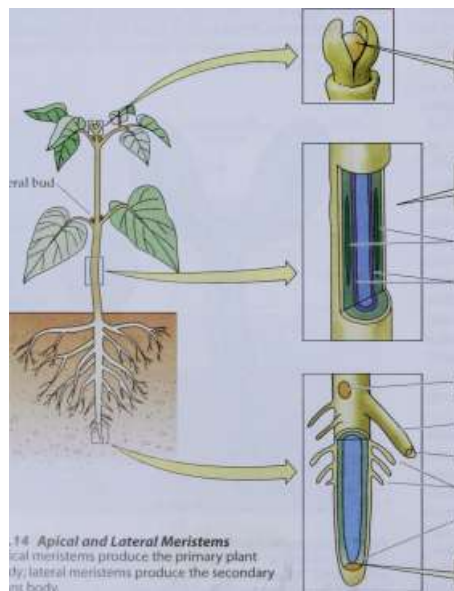
- Apical meristems give rise to the primary plant body, which is the entire body of many plants.
- Lateral meristems give rise to the secondary plant body. The stems and roots of some plants (most obviously trees) form wood and become thick; it is the lateral meristems that give rise to the tissues responsible for this thickening.

apical meristems. Apical meristems are located at the tips of roots and stems, and in buds. They elongate the plant body by producing the cells that subsequently expand and differentiate to form all plant organs (Figure 34.14).

- Shoot apical meristems supply the cells that extend stems and branches, allowing more leaves to form and photosynthesize.
- Root apical meristems supply the cells that extend roots, enabling the plant to "forage" for water and minerals.

Both root and shoot apical meristems give rise to a set of cylindrical primary meristems that produce the primary tissues of the plant body. From the outside to the inside of the root or shoot, which are both cylindrical organs, the primary meristems are the protoderm, the ground meristem,

Lateral bud



Leaf primordia

34.14 Apical and Lateral Meristems

Apical meristems produce the primary plant body; lateral meristems produce the secondary plant body.

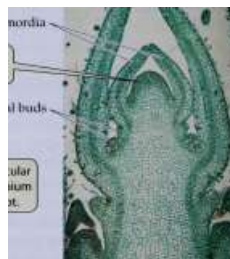
The apical bud contains a shoot apical meristem.

Lateral buds

In woody plants the vascular cambium and cork cambium thicken the stem and root.

Cork cambium

Vascular cambium



Meristem that will form lateral root

Lateral root

Root apical meristem

Root hairs

Root apical meristem

The root apical meristem is just behind the root cap.



and the procambium. They give rise to the three tissue systems as follows:

Apical meristems give rise to

Primary meristems that give rise to

Tissue systems

Root or shoot apical meristem

<E

Protoderm -

Ground meristem-

Procambium -

► Dermal tissue system

Ground tissue system

► Vascular tissue system

Apical meristems are responsible for primary growth, which leads to elongation and organ formation. All plant organs arise ultimately from cell divisions in the apical meristems, followed by cell expansion and differentiation. Primary growth gives rise to the entire body of many plants.

Because meristems can continue to produce new organs, the plant body is much more variable in form than the animal body, whose organs are laid down only once.

lateral meristems. Some roots and stems develop a secondary body—what we commonly refer to as wood and bark. In an oak tree, for example, the secondary body constitutes almost the entire stem and root system. These complex tissues are derived from two lateral meristems: the vascular cambium and the cork cambium (see Figure 34.14).

The vascular cambium is a cylindrical tissue consisting of vertically elongated cells that divide frequently. Toward the inside of the stem or root the dividing cells form new xylem, and toward the outside they form new phloem.

As a tree grows in diameter, the outermost layers of the stem crack and fall off. Without the activity of the cork cambium, this sloughing off of the dermal tissues would expose the tree to potential damage, including excessive water loss or invasion by microorganisms. The cork cambium produces new protective cells, primarily in the outward direction. The walls of these cells become impregnated with suberin, which makes them waterproof. The layer of growth produced by the cork cambium is called the periderm.

Growth in the diameter of stems and roots, produced by the vascular and cork cambia, is called secondary growth. It is the source of wood and bark. Wood is secondary xylem. Bark (periderm plus secondary phloem) is everything external to the vascular cambium.

In some plants, meristems may remain active for years— even centuries. The bristlecone pine mentioned at the beginning of this chapter provides a dramatic example. Such plants grow in size, or at least in diameter, throughout their lives. Recall that this pattern of continuous growth is known as ∞ determinate growth. Determinate growth, which stops at some point, is characteristic of some plant parts, such as leaves, flowers, and fruits, as well as most animals.

In the sections that follow, we'll examine how the various meristems give rise to the plant body.

The root apical meristem gives rise to the root cap and the primary meristems

The root apical meristem produces all the cells that contribute to growth in the length of the root. Some of the

daughter cells from the apical end of the root apical meristem contribute to a root cap, which protects the delicate growing region of the root as it pushes through the soil. Cells of the root cap are often damaged or scraped away and must therefore be replaced constantly. The root cap is also the structure that detects the pull of gravity and thus controls the downward growth of roots.

The daughter cells that are produced at the other end of the meristem elongate and lengthen the root. Following elongation, these cells differentiate, giving rise to the various tissues of the mature root. The growing region above the apical meristem— away from the root cap—comprises the three cylindrical primary meristems that give rise to the three tissue systems of the root: the protoderm, the ground meristem, and the procambium (Figure 34.15).

The apical and primary meristems constitute the zone of cell division, the source of all the cells of the root's primary tissues. Just above this zone is the zone of cell elongation, where the newly formed cells are elongating and thus causing the root to reach farther into the soil. Above this is the zone of cell differentiation, where the cells are taking on specialized forms and functions. These three zones grade imperceptibly into one another; there is some cell division even as far up as the zone of cell differentiation, and some cells differentiate even in the zone of cell division.

The products of the root's primary meristems become root tissues

What are the products of the three primary meristems? The protoderm gives rise to the outer layer of cells—the epidermis—which is adapted for protection of the root and for the absorption of mineral ions and water (Figure 34.16). Epi-

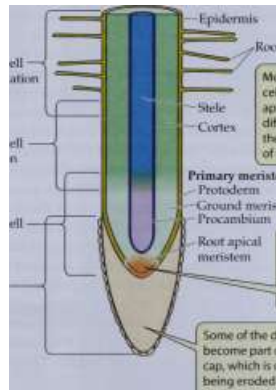
Epidermis

Zone of cell differentiation

Zone of cell elongation

Zone of cell division

Root cap



Root hairs

of the

Most daughter

cells of the root apical meristem differentiate into the primary tissues of the root.

Primary meristems:

Protoderm Ground meristem Procambium

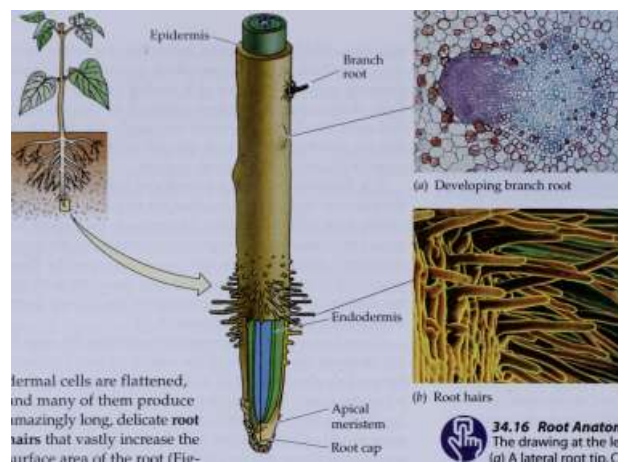
New daughter cells are produced in the root apical meristem.

Some of the daughter cells become part of the root cap, which is constantly being eroded away.

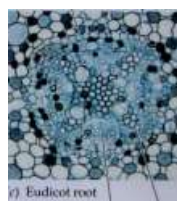
34.15 Tissues and Regions of the Root Tip

Extensive cell division creates the complex structure of the root.

614 CHAPTER THIRTY-FOUR

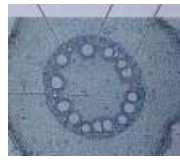


Endodermis Pericycle



3W

(c) Eudicot root Endodermis Phloem Xylem



Pith

dermal cells are flattened, and many of them produce amazingly long, delicate root hairs that vastly increase the surface area of the root (Figure 34.16b). It has been estimated that the root system of a mature rye plant has a total absorptive surface of more than 600 square meters. Root hairs grow out among the soil particles, probing nooks and crannies and taking up water and minerals.

Internal to the epidermis, the ground meristem gives rise to a region of ground tissue that is many cells thick, called the cortex. The cells of the cortex are relatively un-specialized and often function in food storage.

In many plants, but especially in trees, epidermal and sometimes cortical cells form an association with a fungus. This association, called a mycorrhiza, increases the absorption of minerals and water by the plant (see Figure 30.16). Some plant species have poorly developed root hairs or no root hairs. These plants cannot survive unless they develop mycorrhizae that help them with mineral absorption.

Proceeding inward, we come to the endodermis of the root, a single cylindrical layer of cells that is the innermost cell layer of the cortex. The cell walls of the endodermal cells differ markedly from those of the other cortical cells. Endodermal cell walls contain suberin, which forms a waterproof seal wherever it is present. The placement of the seal in just certain parts of the wall enables endodermal cells to control the access of water and dissolved ions to the vascular tissues.

Moving inward past the endodermis, we enter the vascular cylinder, or stele, produced by the procambium. The stele consists of three tissues: pericycle, xylem, and phloem (Figure 34.17). The pericycle consists of one or more layers of relatively undifferentiated cells. It is the tissue within which lateral roots arise (see Figure 34.16f). The pericycle also contributes to secondary growth.

At the very center of the root of a eudicot lies the

lem—seen in cross section as a star with a variable number of points. Between the points are bundles of phloem. In

(d) Monocot root

,\m-

34.16 Root Anatomy

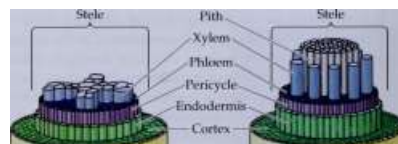
The drawing at the left shows a generalized root structure.

(a) A lateral root tip. Cells in the pericycle divide and the products differentiate, forming the tissues of a lateral root, (b) Root hairs, seen with a scanning electron micrograph. {c,d) The primary root tissues of a eudicot and a monocot. The monocot has a central pith region; the eudicot does not.

monocots, a region of parenchyma cells, called the pith, lies in the center of the root. The pith often stores carbohydrate reserves. It is useful to try picturing these structures in three dimensions (as in Figure 34.17), rather than attempting to understand their functions solely on the basis of two-dimensional cross sections (as in Figure 34.16c, d).

The products of the stem's primary meristems become stem tissues

The shoot apical meristem, like the root apical meristem, forms three primary meristems, which in turn give rise to the three tissue systems. Leaves arise from bulges called leaf



Eudicot root

Monocot root

34.77 The Stele

The distribution of tissues in the stele—the region internal to the endodermis—differs in the roots of eudicots and monocots.

primordia, which form as cells divide on the sides of shoot apical meristems (see Figure 34.14). The growing stem has no cap analogous to the root cap, but the leaf primordia can act as a protective covering.

The plumbing of angiosperm stems differs from that of roots. In a root, the vascular tissue lies deep in the interior, with the xylem at or near the very center. The vascular tissue of a young stem, however, is divided into discrete vascular bundles,

which in eudicots generally form a cylinder but in monocots are seemingly scattered throughout a cross section of the stem (Figure 34.18). Each vascular bundle contains both xylem and phloem.

The stem contains other important tissues in addition to the vascular tissues. Internal to the vascular bundles of eudicots is a storage tissue, the pith, and to the outside lies a similar storage tissue, the cortex. The cortex may contain strengthening collenchyma cells with thickened walls. The pith, the cortex, and the regions between the vascular bundles in eudicots—called pith rays—constitute the ground tissue system of the stem. The outermost cell layer of the young stem is the epidermis, the primary function of which is to minimize the loss of water from the cells within.

Many stems and roots undergo secondary growth

Some stems and roots remain slender and show little or no growth in diameter (secondary growth). However, in many eudicots, stems and roots thicken considerably. This thickening is of great importance and interest because it gives rise to wood and bark, and it makes the support of tall trees possible.

Secondary growth results from the activity of the two lateral meristems: vascular cambium and cork cambium (see Figure 34.14). Vascular cambium consists of cells that divide to produce new (secondary) xylem and phloem cells, while cork cambium produces mainly waxy-walled cork cells.

Initially, the vascular cambium is a single layer of cells

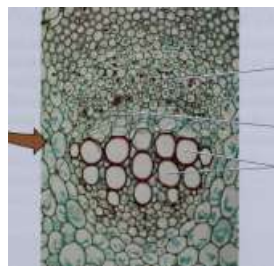
k 34.18 Vascular Bundles in Stems

(a) In eudicots the vascular bundles are arranged in a cylinder, with pith in the center and cortex outside the ring. (b) This scattered arrangement of bundles is typical of monocot stems.

lying between the primary xylem and the primary phloem. The root or stem increases in diameter when the cells of the vascular cambium divide, producing secondary xylem cells toward the inside of the root or stem and producing secondary phloem cells toward the outside (Figure 34.19). In the stems of woody plants, cells of the pith rays between the vascular bundles also divide, forming a continuous cylinder of vascular cambium running the length of the stem. This cylinder in turn gives rise to complete cylinders of secondary xylem (wood) and secondary phloem from which bark will develop.

As the vascular cambium produces secondary xylem and phloem, its principal cell products are vessel elements, supportive fibers, and parenchyma cells in the xylem, and sieve tube members, companion cells, fibers, and parenchyma cells in the phloem. The parenchyma cells in the xylem and phloem store carbohydrate reserves in the stem and root.

Living tissues such as this storage parenchyma must be connected to the sieve tubes of the phloem, or they will starve to death. The connections are provided by vascular rays, which are composed of cells derived from the vascular cambium. The rays, laid down progressively as the cam-



Phloem

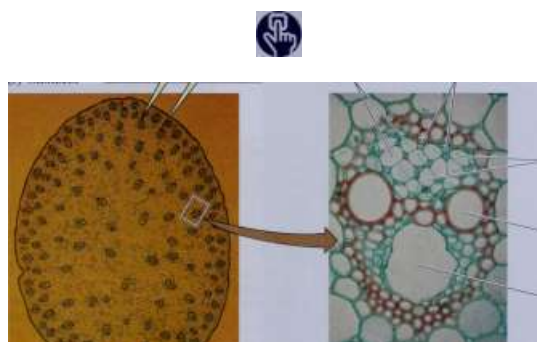
Cambium

Xylem

(b) Monocot

The vascular tissues in stems are organized into bundles.

Eudicot vascular bundle Sieve tube members Companion cells



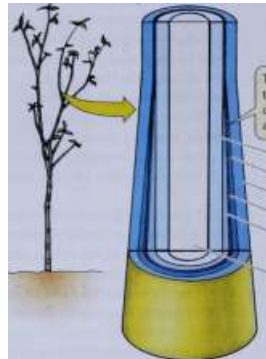
Phloem

Xylem

Air space

Monocot vascular bundle

616 CHAPTER THIRTY-FOUR



m



34.19 Vascular Cambium Thickens Stems and Roots

Stems and roots grow thicker because a thin layer of cells, the vascular cambium, remains meristematic.

The vascular cambium

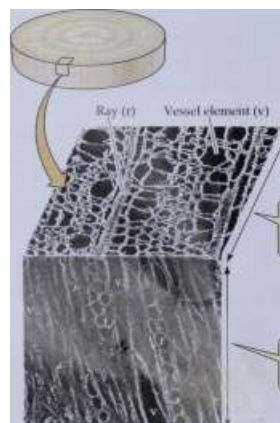
thickens the stem by producing secondary xylem and secondary phloem.

Primary xylem Secondary phloem Primary phloem Vascular cambium Secondary xylem

Pith

bium divides, are rows of living parenchyma cells that run perpendicular to the xylem vessels and phloem sieve tubes (Figure 34.20). As the root or stem continues to increase in diameter, new vascular rays are initiated so that this storage and transport tissue continues to meet the needs both of the bark and of the living cells in the xylem.

The vascular cambium itself increases in circumference with the growth of the root or stem. To do this, some of its cells divide in a plane at right angles to the plane that gives rise to secondary xylem and phloem. The products of each of these divisions lie within the vascular cambium itself and increase its circumference.



^ -J Rays conduct | nutrients horizontally.

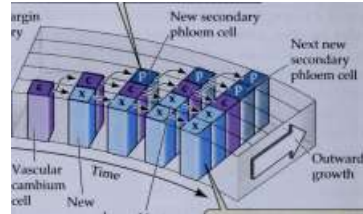
Vessel elements conduct water vertically.

When a vascular cambium cell divides, it produces either a new xylem cell toward the inside of the stem or root, or a new phloem cell toward the outside.

Outer margin of primary xylem

New secondary phloem cell

Next new secondary phloem cell



Vascular

cambium

cell New

secondary xylem cell

Next new secondary xylem cell

Older xylem and phloem cells are pushed farther from the cambium with each division of the cambium.

Only eudicots have a vascular cambium and a cork cambium and thus undergo secondary growth. In the rare cases in which monocots form thickened stems—palm trees, for example—they do so without using vascular cambium or cork cambium. Palm trees have a very wide apical meristem that produces a wide stem, and dead leaf bases also add to the diameter of the stem. Basically, monocots grow in the same way as do other angiosperms that lack secondary growth.

Wood and bark are unique to plants showing secondary growth. These tissues have their own patterns of organization and development.

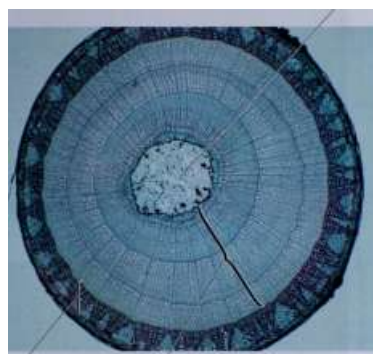
wood. Cross sections of most tree trunks (stems) in temperate-zone forests show annual rings (Figure 34.21), which result from seasonal environmental conditions. In spring, when water is relatively plentiful, the tracheids or vessel elements produced by the vascular cambium tend to be large in diameter and thin-walled. As water becomes less available during the summer, narrower cells with thicker walls are produced, making this summer wood darker and perhaps more dense than the wood formed in spring. Thus each year is usually recorded in a tree trunk by a clearly visible annual ring consisting of one light and one dark layer. Trees in the wet tropics do not lay down such obvious regular rings.

The difference between old and new regions also contributes to the appearance of wood. As a tree grows in di-

34.20 Vascular Rays and Vessel Elements

Wood of the tulip poplar, showing that the orientation of xylem vessels is perpendicular to that of vascular rays. The rays transport sieve tube sap horizontally from the phloem to storage parenchyma cells.

Pith



Secondary phloem

Secondary xylem

34.21 Annual Rings

Rings of secondary xylem are the most noticeable feature of this cross section from a 3-year-old basswood stem.

bark. As secondary growth of stems or roots continues, the expanding vascular tissue stretches and breaks the epidermis and cortex, which ultimately flake away and are lost. Tissue derived from the secondary phloem then becomes the outermost part of the stem. Before the dermal tissues are broken away, cells lying near the surface of the secondary phloem begin to divide and produce layers of cork, a tissue composed of cells with thick walls, waterproofed with suberin. The cork soon becomes the outermost tissue of the stem or root. The dividing cells, derived from the secondary phloem, form a cork cambium. Sometimes the cork cambium produces cells to the inside as well as to the outside; these cells constitute what is known as the phelloderm.

Cork, cork cambium, and phelloderm make up the periderm of the secondary plant body. As the vascular cambium continues to produce secondary vascular tissue, the corky layers are in turn lost, but the continuous formation of cork cambium in the underlying phloem gives rise to new corky layers.

When bark forms on stems and roots, the underlying tissues still need to release carbon dioxide and take up oxygen. Lenticels are spongy regions in the cork of stems and roots that allow such gas exchange (Figure 34.22).

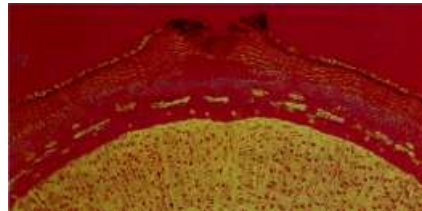
ameter, the xylem toward the center becomes clogged with water-insoluble substances and ceases to conduct water and minerals; this is heartwood and appears darker in color. The portion of the xylem that is actively conducting all water and minerals in the tree is called sapwood and is lighter in color and more porous than heartwood.

The knots that we find attractive in knotty pine but regard as a defect in structural timbers are cross sections of branches: As a trunk grows, the bases of branches become buried in the trunk's new wood and appear as knots when the trunk is cut lengthwise.

Leaf Anatomy Supports Photosynthesis

We can think of roots and stems as important supporting actors that sustain the activities of the real stars of the plant body, the leaves—the organs of photosynthesis. Leaf anatomy is beautifully adapted to carry out photosynthesis and to support photosynthesis by exchanging the gases O_2 and CO_2 with the environment, limiting evaporative water loss, and transporting the products of photosynthesis to the rest of the plant. Figure 34.23f shows a typical eudicot leaf in cross section.

(a)



(b)

■ iMfr tfiifiliI twh 11 Mi" • ■ -.'

34.22 Lenticels Allow Gas Exchange through the Periderm

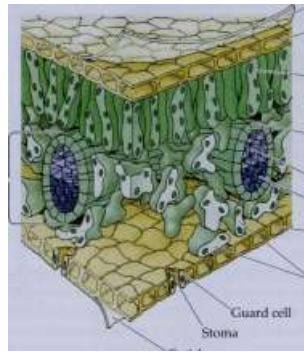
(a) The region of periderm that appears broken open is a lenticel in a year-old elder twig; note the spongy tissue that constitutes the lenticel. (b) The rough areas on the trunk of this Chinese plum tree are lenticels. Most tree species have lenticels much smaller than these.



(a)

(b)

Vein <



Guard cell Stoma Cuticle

Cuticle

Upper epidermis

Palisade

mesophyll

cell

Bundle sheath cell

Xylem

Phloem

Lower epidermis

Spongy

mesophyll

cells

rn



34.23 The Eudicot Leaf

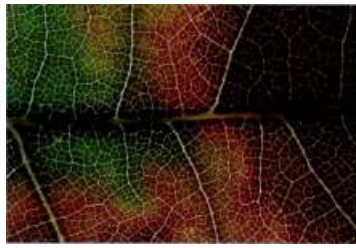
(a) Cross section of a eudicot leaf, (b) The network of fine veins in this maple leaf carries water to the mesophyll cells and carries photosynthetic products away from them, (c) These paired cells on the lower epidermis of a eudicot leaf are guard cells; the gaps between them are stomata, through which carbon dioxide enters the leaf.

Most eudicot leaves have two zones of photosynthesizing parenchyma tissue referred to as mesophyll, which means "middle of the leaf." The upper layer or layers of mesophyll consist of roughly cylindrical cells. This zone is referred to as palisade mesophyll. The lower layer or layers consist of irregularly shaped cells; this zone is called spongy mesophyll. Within the mesophyll is a great deal of air space through which carbon dioxide can diffuse to surround all photosynthesizing cells.

Vascular tissue branches extensively in the leaf, forming a network of veins (Figure 34.23b). Veins extend to within a few cell diameters of all the cells of the leaf, ensuring that the mesophyll cells are well supplied with water and minerals. The products of photosynthesis are loaded into the phloem of the veins for export to the rest of the plant.

Covering the entire leaf is a layer of nonphotosynthetic cells constituting the epidermis. The epidermal cells have an overlying waxy cuticle that is highly impermeable to water. But this impermeability poses a problem: While keeping water in the leaf, the epidermis also keeps carbon dioxide, the other raw material of photosynthesis, out.

The problem of balancing water retention and carbon dioxide availability is solved by an elegant regulatory system that will be discussed in more detail in the next chapter. Guard cells are modified epidermal cells that change their shape, thereby opening or closing pores called stomata, which serve as passageways between the environment and the leaf's interior (Figure 34.23c). When the stomata are open, carbon dioxide can enter and oxygen can leave, but some water can also be lost.



Guard cells

Stomata

(c)



In Chapter 8 we described C₄ plants, which can fix carbon dioxide efficiently even when the carbon dioxide supply in the leaf decreases to a level at which the photosynthesis of C₃ plants is inefficient. One adaptation that helps C₄ plants do this is their modified leaf anatomy, as shown in Figure 8.19. Notice that the photosynthetic cells in the C₄ leaf are grouped around the veins in concentric layers, forming an outer mesophyll layer and an inner bundle sheath. These layers each contain different types of chloroplasts, leading to the biochemical division of labor described in Chapter 8.

Leaves receive water and mineral nutrients from the roots by way of the stems. In return, the leaves export products of photosynthesis, providing a supply of chemical energy to the rest of the plant body. And, as we have just seen, leaves exchange gases, including water vapor, with the environment by way of the stomata. All three of these processes will be considered in detail in the next chapter.

Chapter Summary

Vegetative Organs of the Flowering Plant Body

- Monocots typically have a single cotyledon, narrow leaves with parallel veins, flower parts in threes or multiples of three, and stems with scattered vascular bundles.
- Eudicots typically have two cotyledons, broad leaves with netlike veins, flower parts in fours or fives, and vascular bundles in a ring. Flowering plants that are neither monocots nor eudicots are generally similar in structure to eudicots. Review Figure 34.1
- The vegetative organs of flowering plants are roots, which form a root system, and stems and leaves, which form a shoot system. Review Figure 34.2

THE PLANT BODY 619

- Roots anchor the plant and take up water and minerals.
- Stems bear leaves and buds. Lateral buds form branches. Apical buds produce cells that contribute to the elongation of the stem.
- Leaves are responsible for most photosynthesis, for which their flat blades, oriented perpendicular to the sun's rays, are well adapted. Review Figure 34.5

Plant Cells

- The walls of plant cells have a structure that often corresponds to the special functions of the cell. The walls of individual cells are separated by a middle lamella common to two neighboring cells; each cell also has its own primary wall. Review Figure 34.6
- Some cells produce a thick secondary wall. Adjacent cells are connected by plasmodesmata that extend through both cell walls. Review Figures 34.7, 34.8
- Parenchyma cells have thin walls. Many parenchyma cells store starch or lipids; some others carry out photosynthesis. Review Figure 34.9a
- Collenchyma cells provide flexible support. Review Figure 34.9b
- Sclerenchyma cells provide strength and function when dead. Review Figure 34.9c, d

► Tracheids and vessel elements are xylem cells that conduct water and minerals after the cells die. Review Figures 34.9e, /, 34.10

► Sieve tube members are the conducting cells of the phloem. Their activities are often controlled by companion cells. Review Figure 34.11

Plant Tissues and Tissue Systems

► Three tissue systems extend throughout the plant body. The vascular tissue system, consisting of xylem and phloem, conducts water, minerals, and the products of photosynthesis. The dermal tissue system protects the body surface. The ground tissue system produces and stores food materials and performs other functions. Review Figure 34.12

Forming the Plant Body

► The apical-basal pattern and the radial pattern are parts of the plant body plan; they arise through orderly development. Review Figure 34.13

► The plant body is modular, and the growth of stems and roots is indeterminate. Leaves, flowers, and fruits show determinate growth.

► Meristems are localized regions of cell division. A hierarchy of meristems generates the plant body.

► Apical meristems at the tips of stems and roots produce the primary growth of those organs. Review Figure 34.14

► Shoot apical meristems and root apical meristems give rise to primary meristems: the protoderm, the ground meristem, and the procambium. Review Figure 34.15

► In some plants, the products of primary growth constitute the entire plant body. Many other plants show secondary growth. Two lateral meristems, the vascular cambium and cork cambium, are responsible for secondary growth. Review Figure 34.14

► The young root has an apical meristem that gives rise to the root cap and to the three primary meristems, which in turn produce the three tissue systems. The protoderm produces the dermal tissue system, the ground meristem produces the ground tissue system, and the procambium produces the vascular tissue system. Review Figure 34.15

► Root tips have three overlapping zones: the zone of cell division, the zone of cell elongation, and the zone of cell differentiation. Review Figure 34.15

► The dermal tissue system consists of the epidermis, part of which forms the root hairs that are responsible for absorbing water and minerals. Review Figure 34.16

► The ground tissue system of a young root is the cortex, whose innermost cell layer, the endodermis, controls access to the stele.

► The stele, consisting of the pericycle, xylem, and phloem, is the root's vascular tissue system. Lateral roots arise in the pericycle. Monocot roots have a central pith region. Review Figure 34.17

► The shoot apical meristem also gives rise to three primary meristems, with roles similar to their counterparts in the root. Leaf primordia on the sides of the apical meristem develop into leaves.

► The vascular tissue in young stems is divided into vascular bundles, each containing both xylem and phloem. Pith occupies the center of the eudicot stem. Cortex lies to the outside of the vascular bundles in eudicots, with pith rays lying between the vascular bundles. Review Figure 34.18

► Many eudicot stems and roots show secondary growth, in which vascular and cork cambia give rise to secondary xylem and secondary phloem. As secondary growth continues, the products are wood and bark. Review Figure 34.19

► The vascular cambium lays down layers of secondary xylem and phloem. Living cells within these tissues are nourished by vascular rays. Review Figure 34.20

► The periderm consists of cork, cork cambium, and phello-derm, all pierced at intervals by lenticels that allow gas exchange.

Leaf Anatomy Supports Photosynthesis

► The photosynthetic tissue of a leaf is called mesophyll. Veins bring water and minerals to the mesophyll and carry the products of photosynthesis to other parts of the plant body.

► A waxy cuticle prevents water loss from the leaf, but is impermeable to carbon dioxide. Guard cells control the opening of stomata, openings in the leaf that allow CO_2 to enter but also allow some water to escape. Review Figure 34.23

For Discussion

1. When a young oak was 5 m tall, a thoughtless person carved his initials in its trunk at a height of 1.5 m above the ground. Today that tree is 10 m tall. How high above the ground are those initials? Explain your answer in terms of the manner of

plant growth.

2. Consider a newly formed sieve tube member in the secondary phloem of an oak tree. What kind of cell divided to produce the sieve tube member? What kind of cell divided to produce that parent cell? Keep tracing back until you arrive at a cell in the apical meristem.
3. Distinguish between sclerenchyma cells and collenchyma cells in terms of structure and function.
4. Distinguish between primary and secondary growth. Do all angiosperms undergo secondary growth? Explain.
5. What anatomical features make it possible for a plant to retain water as it grows? Describe the plant tissues and how and when they form.



Transport in Plants



About 40 years ago the biologist Per Scholander was studying water movement to the top of an 80-meter Douglas fir. To collect samples rapidly from the treetop, he hired a sharpshooter, who aimed a high-powered rifle at a twig high in the tree and fired. From high above, a twig fluttered to the ground, and Scholander quickly inserted it into an instrument for measuring tension in the xylem sap. As we will soon see, Scholander's measurements increased our understanding of how water and minerals reach the tops of tall trees.

The water and minerals in a plant's xylem must be transported to the entire shoot system, all the way to the highest leaves and apical buds. Carbohydrates produced in all the leaves, including the highest, must be translocated to all the living nonphotosynthetic parts of the plant. Before we consider the mechanisms underlying these processes, we should consider two questions: How much water is transported? And how high can water be transported?

In answer to the first question, consider the following example: A single maple tree 15 meters tall was estimated to have some 177,000 leaves, with a total leaf surface area of 675 square meters—half again the area of a basketball court. During a summer day, that tree lost 220 liters of water per hour to the atmosphere by evaporation from the leaves. To prevent wilting, the xylem needed to transport 220 liters of water from the roots to the leaves every hour. (By comparison, a 50-gallon drum holds 189 liters.)

The second question can be rephrased: How tall are the tallest trees? The tallest gymnosperms, the coast redwoods—*Sequoia sempervirens*—exceed 110 meters in height, as do the tallest angiosperms, the Australian *Eucalyptus regnans*. Any successful explanation of water transport in the xylem must account for transport to these great heights.

In this chapter we consider the uptake and transport of water and minerals by plants, the control of evaporative water loss through the stomata, and the translocation of substances in the phloem.

A Long Way to the Top

Water and minerals must defy gravity and climb over 80 meters to reach the top branches of these Douglas firs (*Pseudotsuga menziesii*).

Uptake and Transport of Water and Minerals

Terrestrial plants obtain both water and mineral nutrients from the soil, usually by way of their roots. You already know that water is one of the ingredients required for carbohydrate production by photosynthesis in the leaves. Water is also essential for transporting solutes, for cooling the plant, and for developing the internal pressure that supports the plant body.

How do leaves high in a tree obtain water from the soil? What are the mechanisms by which water and mineral ions enter the plant body through the roots and ascend as sap in the xylem? Because neither water nor minerals can move through the plant into the xylem without crossing at least



one plasma membrane, we focus first on osmosis. Then we examine the uptake of mineral ions, and follow the pathway by which both water and minerals move through the root to gain entry to the xylem.

Water moves through a membrane by osmosis

Osmosis, the movement of water through a membrane in accordance with the laws of diffusion, was described in Chapter 5. The solute potential (osmotic potential) of a solution is a measure of the effect of dissolved solutes on the osmotic behavior of the solution. The greater the solute concentration of a solution, the more negative its solute potential, and the greater the tendency of water to move into it from another solution of lower solute concentration (and less negative solute potential). For osmosis to occur, the two solutions must be separated by a membrane permeable to water but relatively impermeable to the solute. Recall, too, that osmosis is a passive process—energy is not required.

Unlike animal cells, plant cells are surrounded by a relatively rigid cell wall. As water enters a plant cell, the entry of more water is increasingly resisted by an opposing pressure potential (turgor pressure), owing to the rigidity of the wall. As more and more water enters, the pressure potential becomes greater and greater.

Pressure potential is a hydraulic pressure analogous to the air pressure in an automobile tire; it is a real pressure that can be measured with a pressure gauge. Cells with walls do not burst when placed in pure water; instead, water enters by osmosis until the pressure potential exactly balances the solute potential. At this point, the cell is turgid; that is, it has a high pressure potential.

The overall tendency of a solution to take up water from pure water, across a membrane, is called its water potential, represented as ψ , the Greek letter psi (Figure 35.1). The water potential

is simply the sum of the (negative) solute potential (ψ_s) and the (usually positive) pressure potential (ψ_p):

$$\psi = \psi_s + \psi_p$$

For pure water under no applied pressure, all three of these parameters are zero.

We can measure solute potential, pressure potential, and water potential in megapascals (MPa), a unit of pressure. (Atmospheric pressure is about 0.1 MPa, or 14.7 pounds per square inch; typical pressure in an automobile tire is about 0.2 MPa.)

In all cases in which water moves between two solutions separated by a membrane, the following rule of osmosis applies: Water always moves across a differentially permeable membrane toward the region of more negative water potential.

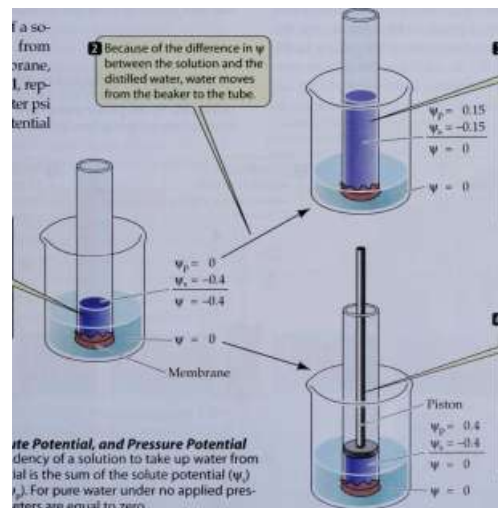
Osmotic phenomena are of great importance to plants. The structure of many plants is maintained by the pressure potential of their cells; if the pressure potential is lost, a plant wilts. Within living tissues, the movement of water from cell to cell by osmosis follows a gradient of water potential. Over longer distances, in open tubes such as xylem vessels and phloem sieve tubes, the flow of water and dissolved solutes is driven by a gradient in pressure potential. The movement of a solution due to a difference in pressure potential between two parts of a plant is called bulk flow.

Uptake of mineral ions requires transport proteins

Mineral ions, which carry electric charges, cannot move across a plasma membrane unless they are aided by trans-

§j Because of the difference in ψ between the solution and the distilled water, water moves from the beaker to the tube.

If the solution in the tube has a negative solute potential (ψ_s) due to the presence of dissolved solutes; its $\psi_p = 0$; thus its ψ is negative. The beaker contains distilled water ($\psi = 0$). The two liquids are not at equilibrium.



Water entering the tube dilutes the solution, making its ψ_s less negative. As the solution rises in the tube, pressure potential (ψ_p) builds up until it balances the ψ_s . This pressure corresponds to turgor pressure in plants. At equilibrium, ψ in the solution is equal to ψ in the beaker.

35.1 Water Potential, Solute Potential, and Pressure Potential

Water potential (ψ) is the tendency of a solution to take up water from pure water. The water potential is the sum of the solute potential (ψ_s) and the pressure potential (ψ_p). For pure water under no applied pressure, all three of these parameters are equal to zero.

A piston resists the entry of water, as does the wall of a plant cell. The solution in the tube is not diluted, so its ψ_s does not change. However, the system is not initially at equilibrium. Enough water squeezes in to raise ψ_p until equilibrium is reached, with equal water potentials.

622 CHAPTER THIRTY-FIVE

transport proteins. (You may wish to review the description of transport proteins in Chapter 5.) When the concentration of these charged ions in the soil is greater than that in the plant, ion channels and carrier proteins can move them into the plant by facilitated diffusion.

The concentrations of some ions in the soil solution, however, are lower than those required inside the plant. Thus the plant must take up these ions against a concentration gradient. Electric potential also plays a role in this process: To move a negatively charged ion into a negatively charged region is to move it against an electrical gradient. The combination of concentration and electrical gradients is called an electrochemical gradient. Uptake against an electrochemical gradient is active transport, an energy-requiring process, depending on cellular respiration for ATP. Active transport, of course, requires specific carrier proteins.

Unlike animals, plants do not have a sodium-potassium pump for active transport. Rather, plants have a proton pump, which uses energy obtained from ATP to move protons out of the cell against a proton concentration gradient (Figure 35.2a). Because protons (H^+) are positively charged, their accumulation on one side of a membrane has two results:

- The region outside the membrane becomes positively charged with respect to the region inside.
- A proton concentration gradient develops.

Each of these results has consequences for the movement of other ions. Because of the charge difference across the membrane, the movement of positively charged ions, such as potassium (K^+), into the cell through their membrane channels is enhanced. These positive ions move into the now more negatively charged interior of the cell by facilitated diffusion (Figure 35.2b). In addition, the proton concentration gradient can be harnessed to drive secondary active transport, in which negatively charged ions such as

chloride (Cl^-) are moved into the cell against an electrochemical gradient by a symport protein that couples their movement with that of H^+ (Figure 35.2c). In sum, there is vigorous traffic of ions across plant membranes.

The proton pump and the coordinated activities of other membrane transport proteins cause the interior of a plant cell to be strongly negative with respect to the exterior. Such a difference in charge across a membrane is called a membrane potential. Biologists can measure the membrane potential of a plant cell with microelectrodes, just as they can measure similar charge differences in neurons (nerve cells) and other animal cells. Most plant cells have a membrane potential of at least -120 millivolts, and they maintain it at this level. The membrane potential difference affects the movements of mineral ions into and out of cells.

Water and ions pass to the xylem by way of the apoplast and symplast

Mineral ions enter and move through plants in various ways. Where bulk flow of water is occurring, dissolved minerals are carried along in the stream. Where water is moving more slowly, minerals move by diffusion. At certain points, where plasma membranes are being crossed, some mineral ions are moved by active transport. One such point is the surface of a root hair, where mineral ions first enter the cells of the plant. Later, within the stele, the ions must cross a plasma membrane before entering the lifeless cells of the xylem.

The movement of ions across membranes can also result in the movement of water. Water moves into a root because the root has a more negative water potential than does the

35.2 The Proton Pump and Its Effects

The buildup of hydrogen ions transported across the plasma membrane by the proton pump {a} triggers the movement of both cations (b) and anions (c) into the cell.

(«)

Q A proton pump generates differences in H^+ concentration and electric potential across the membrane.

Extracellular space

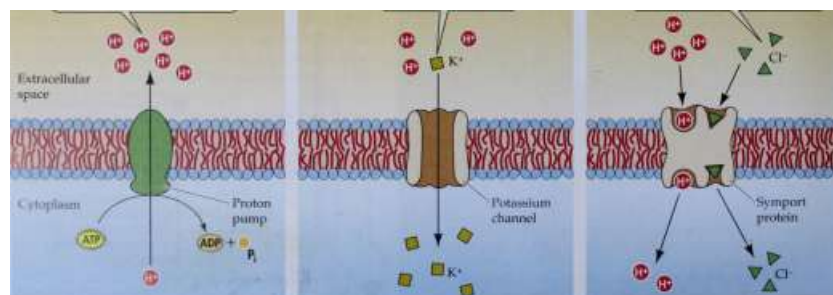
© © ©

(b)

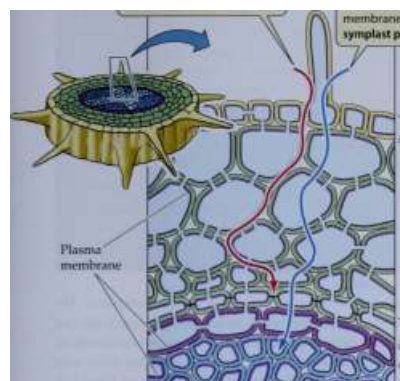
The difference in electric potential causes cations such as K^+ to enter the cell.

(c)

Symport couples the diffusion of H^+ to the transport (against a concentration gradient) of anions such as Cl^- into the cell.



Water travels through cell walls and intercellular spaces in the apoplast path.



Water crosses a plasma membrane and enters the symplast path.

(j» Epidermis

• Endodermis

j- Pericycle

Tracheary elements

soil solution. Water moves from the cortex of the root into the stele (which is where the vascular tissues are located) because the stele has a more negative water potential than does the cortex.

Water and minerals from the soil may pass through the dermal and ground tissues to the stele via two pathways: the apoplast and the symplast. Plant cells are surrounded by cell walls that lie outside the plasma membrane, and intercellular spaces (spaces between cells) are common in many tissues. The walls and intercellular spaces together constitute the apoplast (from the Greek for "away from living material"). The apoplast is a continuous meshwork through which water and

dissolved substances can flow or diffuse without ever having to cross a membrane (Figure 35.3). Movement of materials through the apoplast is thus unregulated.

The remainder of the plant body is the symplast (from the Greek for "together with living material"). The symplast is the portion of the plant body enclosed by membranes—the continuous cytoplasm of the living cells, connected by plasmodesmata (see Figure 35.3). The selectively permeable plasma membranes of the cells control access to the symplast, so movement of water and dissolved substances into the symplast is tightly regulated.

Water and minerals can pass from the soil solution through the apoplast as far as the endodermis, the innermost layer of the cortex. The endodermis is distinguished from the rest of the ground tissue by the presence of Casparian strips. These waxy, suberin-containing structures impregnate the endodermal cell wall and form a belt surrounding the endodermal cells. The Casparian strips act as a gasket that prevents water and ions from moving between the cells (Figure 35.4).



35.3 Apoplast and Symplast

The plant cell walls and intercellular spaces constitute the apoplast. The symplast comprises the living cells, which are connected by plasmodesmata. To enter the symplast, water and solutes must pass through a plasma membrane. No such selective barrier limits movement through the apoplast.

The Casparian strips of the endodermis thus completely separate the apoplast of the cortex from the apoplast of the stele. They do not obstruct the outer or inner faces of the endodermal cells. Accordingly, water and ions can enter the stele only by way of the symplast—that is, by entering and passing through the cytoplasm of the endodermal cells. Thus transport proteins in the membranes of these cells determine which mineral ions $I. ST$ pass into the stele, and at what rates. This is one of

one of several ways in which plants regulate their chemi-

cal composition and ensure an appropriate balance of their constituents. This balance is essential to plant life. Once they have passed the endodermal barrier, water and minerals leave the symplast. Parenchyma cells in the pericycle or xylem help mineral ions move back into the apoplast. Some of these parenchyma cells, called transfer cells, are structurally modified for transporting mineral ions from their cytoplasm (part of the symplast) into their cell walls (part of the apoplast). The wall that receives the transported ions has many knobby extensions projecting into the transfer cell, increasing the surface area of the plasma membrane, the

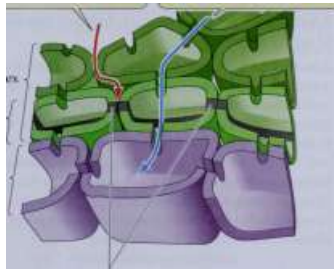
Casparian strips prevent water in the apoplast from passing between the endodermal cells and into the stele.

Water must first enter the living endodermal cells; by entering the symplast, it can evade the Casparian strips.

Cortex

Endodermis

Pericycle (stele)



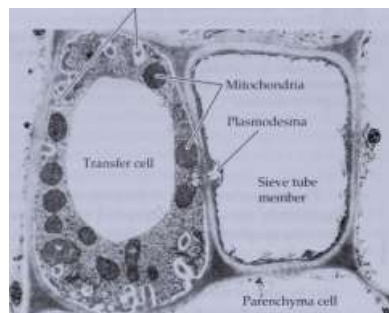
Casparian strips

35.4 Casparian Strips

Suberin-impregnated Casparian strips prevent water and ions from moving between the endodermal cells.

624 CHAPTER THIRTY-FIVE

\\ .ill extensions



35.5 A Transfer Cell

Three walls of this transfer cell in a pea leaf have knobby extensions that face the cells from which the transfer cell imports solutes. This transfer cell exports the solutes to the neighboring sieve tube member.

number of transport proteins, and thus the rate of transport (Figure 35.5).

Transfer cells also have many mitochondria that produce the ATP needed to power the active transport of mineral ions. As mineral ions move into the solution in the walls, the water potential of the wall solution (apoplast) becomes more negative; thus water moves out of the cells and into the apoplast by osmosis. Active transport of ions moves the ions directly, and water follows passively. The end result is that water and minerals end up in the xylem, where they constitute the xylem sap.

We have just seen that proteins regulate the movement of ions across membranes. We shall now see that even water movement itself is regulated by proteins.

Aquaporins control the rate, but not the direction, of water movement

Aquaporins are membrane channel proteins through which water can traverse a membrane without interacting with the hydrophobic environment of its phospholipid bilayer. These proteins, important in both plants and animals, allow water to move rapidly from environment to cell and from cell to cell. The permeability of some aquaporins is subject to regulation, changing the rate of osmosis across the membrane. However, water movement through aquaporins is always passive, so the direction of water movement is unchanged by alterations in aquaporin permeability.

Transport of Water and Minerals in the Xylem

So far in this chapter we've described the movement of water and minerals into plant roots and their entry into the root xylem. Now we will consider how xylem sap moves

throughout the remainder of the plant. Let's first consider some early ideas about the ascent of sap and then turn to our current understanding of how it works. We'll describe the experiments that ruled out some early models as well as some evidence in support of the current model—and we'll find out what Per Scholander's sharpshooter was up to in the story that opened this chapter.

Experiments ruled out some early models of transport in the xylem

Some of the earliest attempts to explain the rise of sap in the xylem were based on a hypothetical pumping action by living cells in the stem, which pushed the sap upward. However, experiments conducted and published in 1893 by the German botanist Eduard Strasburger definitively ruled out such models.

Strasburger worked with trees about 20 meters tall. He sawed them through at their bases and plunged the cut ends into buckets containing solutions of poisons such as picric acid. The solutions rose through the trunks, as was readily evident from the progressive death of the bark higher and higher up. When the solutions reached the leaves, the leaves died, too, at which point the solutions stopped being transported (as shown by the liquid levels in the buckets, which stopped dropping).

This simple experiment established three important points:

- ▶ Living, "pumping" cells were not responsible for the upward movement of the solutions, because the solutions themselves killed all living cells with which they came in contact.
- ▶ The leaves play a crucial role in transport. As long as they were alive, solutions continued to be transported upward; when the leaves died, transport ceased.
- ▶ Transport was not caused by the roots, because the trunks had been completely separated from the roots.

Root pressure does not account for xylem transport

In spite of Strasburger's observations, some plant physiologists turned to a model of transport based on root pressure—pressure exerted by the root tissues that would force liquid up the xylem. The basis for root pressure is a higher solute concentration, and accordingly a more negative water potential, in the xylem sap than in the soil solution. This negative potential draws water into the stele; once there, the water has nowhere to go but up.

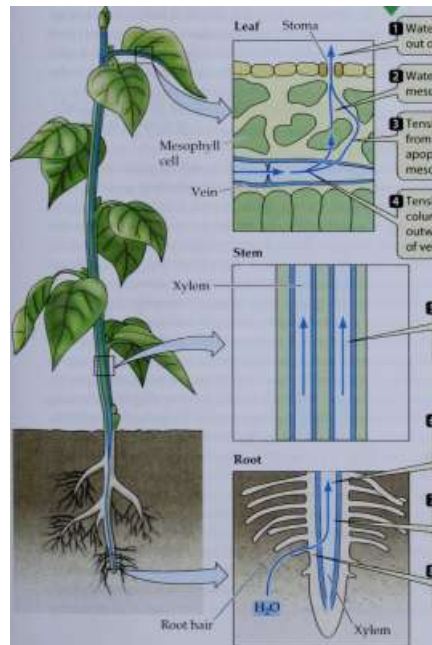
There is good evidence for root pressure—for example, the phenomenon of guttation, in which liquid water is forced out through openings in the leaves (Figure 35.6). Guttation occurs only under conditions of high atmospheric humidity and plentiful water in the soil, which occur most commonly at night. Root pressure is also the source of the sap that oozes from the cut stumps of some plants, such as *Coleus*, when their tops are removed. Root pressure, however, cannot account for the ascent of sap in trees.

Root pressure seldom exceeds 0.1–0.2 MPa (one or two times atmospheric pressure). If root pressure were driving



sap up the xylem, we would observe a positive pressure potential in the xylem at all times. In fact, as we are about to see, the xylem sap is under tension—a negative pressure potential—when it is ascending. Furthermore, as Stras-

n^{ai}



Water vapor diffuses out of the stomata.

Water evaporates from mesophyll cell walls.

Tension pulls water from the veins into the apoplast about the mesophyll cells.

Tension pulls the water column upward and outward in the xylem of veins in the leaves.

35.6 Guttation

Root pressure is responsible for forcing water through openings in the tips of this strawberry leaf.

burger had already shown, materials can be transported upward in the xylem even when the roots have been removed. If the roots aren't pushing the xylem sap upward, what does cause it to rise?

The transpiration-cohesion-tension mechanism accounts for xylem transport

The obvious alternative to pushing is pulling: The leaves pull the xylem sap upward. Transpiration, the evaporative loss of water from the leaves, generates a pulling force (tension) on the water in the apoplast of the leaves. Hydrogen bonding between water molecules makes the sap in the xylem cohesive enough to withstand the tension and rise by bulk flow. Let's see how this transpiration-cohesion-tension mechanism works.

We'll start with transpiration. Water vapor diffuses from the intercellular spaces of the leaf, by way of the stomata, to the outside air because the water vapor concentration is greater inside the leaf than outside. Where did this water vapor come from? As water vapor diffuses out of the leaf, more water evaporates from the moist walls of the mesophyll cells (Figure 35.7). Evaporation of water from the thin film surrounding the cell wall causes the film to shrink into the cellulose meshwork of the wall. The surface of the film curves where the water retreats into microscopic pores. The surface tension of the curved surfaces generates a tension—a negative pressure potential, a pull—in the film. The tension increases as more water leaves the film. This tension is what causes the bulk flow of water all the way from the roots.

The tension in the mesophyll draws water from the vessels or tracheids in the xylem of the nearest vein. The water, with its dissolved solutes, moves by bulk flow through the apoplast. The removal of water from the xylem of the veins establishes tension on the entire column of water contained within the xylem, so the column is drawn upward all the way from the roots.

The ability of water to be pulled upward through tiny tubes results from the remark-

Tension pulls the water column upward in the xylem of the stem

(^Tension pulls the water column upward in the xylem of the root

Q Water molecules form a cohesive column.

Water moves into the stele by osmosis.

35.7 Water Transport in Plants

Evaporation from surface cells, tension generated by the curvature of the shrinking surface film, and the cohesive nature of water molecules all account for the bulk flow of water from the soil to the atmosphere.

able cohesiveness of water—the tendency of water molecules to cohere to one another through hydrogen bonding. The narrower the tube, the greater the tension the water column can withstand without breaking. The integrity of the column is also maintained by the adhesion of water to the cell walls. In the tallest trees, such as a 100-meter redwood, the difference in pressure potential between the top and the bottom of the column may be as great as 3 MPa. The cohesiveness of water in the xylem is great enough to withstand even that great a tension.

In summary, the key elements of water transport in the xylem are:

- ▶ Transpiration, followed by evaporation from the moist cell walls in the leaves, resulting in...
- ▶ tension in the remainder of the xylem's water owing to the...
- ▶ cohesion of water, which pulls up more water to replace water that has been lost.

These elements require no work—no expenditure of energy—on the part of the plant. At each step between soil and atmosphere, water moves passively to a region with a more strongly negative water potential. Dry air has the most negative water potential (-95 MPa at 50% relative humidity), and the soil solution has the least negative water potential (between -0.01 and -3 MPa). Xylem sap has a water potential more negative than that of cells in the root, but less negative than that of mesophyll cells in the leaf.

Mineral ions contained in the xylem sap rise passively with the solution as it ascends from root to leaf. In this way the nutritional needs of the shoot are met. Some of the mineral elements brought to the leaves are subsequently redistributed to other parts of the plant by way of the phloem, but the initial delivery from the roots is through the xylem.

In addition to promoting the transport of minerals, transpiration contributes to temperature regulation. As water evaporates from mesophyll cells, heat is taken up from the cells, and the leaf temperature drops. This cooling effect is important in enabling plants to live in hot environments. A farmer can hold a leaf between thumb and forefinger to estimate its temperature; if the leaf doesn't feel cool, that means that transpiration is not occurring, so it must be time to water.

A pressure bomb measures tension in the xylem sap

The transpiration-cohesion-tension model can be true only if the column of sap in the xylem is under tension (negative pressure potential). The most elegant demonstrations of this tension, and of its adequacy to account for the ascent of sap in tall trees, were performed by Per Scholander. He measured tension in stems with an instrument called a pressure bomb.

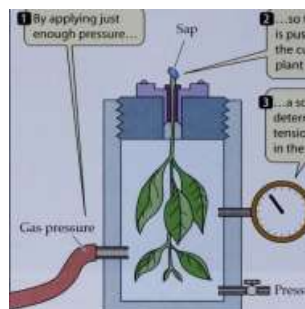
The principle of the pressure bomb is as follows: Consider a stem in which the xylem sap is under tension. If the stem is cut, the sap pulls away from the cut, into the stem. Now the stem is placed in a device called a pressure bomb,

RESEARCH METHOD

t

By applying just enough pressure...

Sap



...so that xylem sap is pushed back to the cut surface of a plant sample...

...a scientist can determine the tension on the sap in the living plant.

Pressure gauge

Pressure release valve

35.8 A Pressure Bomb

The amount of tension on the sap in different types of plants can be measured with this laboratory device.

in which the pressure may be raised. The cut surface remains outside the bomb. As pressure is applied to the plant parts within the bomb, the xylem sap is forced back to the cut surface. When the sap first becomes visible again at the cut surface, the pressure in the bomb is recorded. This pressure is equal in magnitude but opposite in sign to the tension (negative pressure potential) originally present in the xylem (Figure 35.8).

Scholander used the pressure bomb to study dozens of plant species, from diverse habitats, growing under a variety of conditions. In all cases in which xylem sap was ascending, it was found to be under tension. The tension disappeared in some of the plants at night, when transpiration ceased. In developing vines, the xylem sap was under no tension until leaves formed. Once leaves developed, transport in the xylem began, and tensions were recorded.

Suppose you wanted to measure tensions in the xylem at various heights in a large tree, to confirm that the tensions are sufficient to account for the rate at which sap is moving up the trunk. How would you obtain stem samples for measurement? Scholander used surveying instruments to determine the heights of particular twigs and then had a sharpshooter shoot the twigs from the tree with a high-powered rifle. As quickly as the twigs fell to the ground, Scholander inserted them in the pressure bomb and recorded their xylem tension. In every case, the differences in tensions at different heights were great enough to keep the xylem sap ascending.

Although transpiration provides the impetus for transport of water and minerals in the xylem, it also results in the loss of tremendous quantities of water from the plant. How do plants control this loss?

Transpiration and the Stomata

The epidermis of leaves and stems minimizes transpirational water loss by secreting a waxy cuticle, which is impermeable to water. However, the cuticle is also impermeable to carbon dioxide. This poses a problem: How can the leaf balance its need to retain water with its need to obtain carbon dioxide for photosynthesis?

Plants have evolved an elegant compromise in the form of stomata (singular stoma), or gaps, in the epidermis. A pair of specialized epidermal cells called guard cells controls the opening and closing of each stoma (Figure 35.9a). When the stomata are open, carbon dioxide can enter the leaf by diffusion, but water vapor is also lost in the same way. Closed stomata prevent water loss, but also exclude carbon dioxide from the leaf.

Most plants open their stomata only when the light intensity is sufficient to maintain a moderate rate of photosynthesis. At night, when darkness precludes photosynthesis, the stomata remain closed; no carbon dioxide is needed at this time, and water is conserved. Even during the day, the stomata close if water is being lost at too great a rate.

The stoma and guard cells in Figure 35.9a are typical of eudicots. Monocots typically have specialized epidermal cells associated with their guard cells. The principle of operation, however, is the same for both monocot and eudicot stomata. In what follows, we describe the regulation and mechanism of stomatal opening, the normal cycle of opening and closing, and the modified cycle used by some plants that live in dry or saline environments.

The guard cells control the size of the stomatal opening

Light causes the stomata of most plants to open, admitting carbon dioxide for photosynthesis. Another cue for stomatal opening is the level of carbon dioxide in the spaces inside the leaf. A low level favors opening of the stomata, thus allowing the uptake of more carbon dioxide.

Water stress is a common problem for plants, especially on hot, sunny, windy days. Plants have a protective response to these conditions, using the water potential of the mesophyll cells as a cue. Even when the carbon dioxide level is low and the sun is shining, if the mesophyll is too dehydrated—that is, if the water potential of the mesophyll is too negative—the mesophyll cells release a plant hormone called abscisic acid. Abscisic acid acts on the guard cells, causing them to close the stomata and prevent further drying of the leaf. This response reduces the rate of photosynthesis, but it protects the plant.

The increasing internal concentration of potassium ions makes the water potential of the guard cells more negative. Water enters the guard cells by osmosis, increasing their pressure potential. Their cell walls contain cellulose microfibrils that cause the cells to respond to this increase by changing their shapes so that a gap—the stoma—appears between them.

The stoma closes by the reverse process when the proton pump is no longer active. Potassium ions diffuse passively

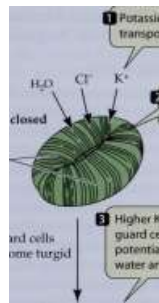
(a)



(b)

Stoma closed

Guard cells



Potassium ions are actively transported into the guard cells.

Cellulose microfibrils control stretching of guard cells.

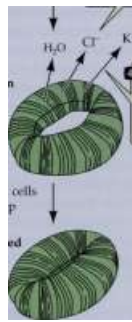
Guard cells become turgid

Higher K^+ and Cl^- concentrations give guard cells a more negative water potential, causing them to take up water and stretch, opening the stoma.

Stoma open

Guard cells go limp

Stoma closed



As K^+ diffuses passively out of the guard cells, water follows by osmosis, the guard cells go limp, and the stoma closes.

35.9 Stomata

(a) A scanning electron micrograph of a gaping stoma between two sausage-shaped guard cells, (b) Potassium ion (K^+) concentrations and water potential control the opening and closing of stomata. Negatively charged ions traveling with K^+ maintain electrical balance and contribute to the changes in osmotic potential that open and close the stomata.

out of the guard cells, water follows by osmosis, the pressure potential decreases, and the guard cells sag together and seal off the stoma. Negatively charged chloride ions and organic ions also move out of the guard cells with the potassium ions, maintaining electrical balance and contributing to the change in the solute potential of the guard cells.

628 CHAPTER THIRTY-FIVE

35.70 Light-Induced Proton Pumping in a Guard Cell Membrane

This graph shows a trace of the tiny electric current that results from the flow of protons across the plasma membrane of a guard cell when it is exposed briefly to blue light.

What drives the opening and closing of the stomata? Certain wavelengths of blue light, absorbed by a pigment in the guard cell plasma membrane, activate a proton pump, which actively transports protons (H^+) out of the guard cells and into the surrounding epidermis (Figure 35.10). The resulting proton gradient drives the accumulation of potassium ions (K^+) (Figure 35.9b) in the guard cell.

Antitranspirants decrease water loss

Stomata are the referees of a compromise between the admission of CO_2 for photosynthesis and the loss of water by transpiration. Farmers would like their crops to transpire less, thus reducing the need for irrigation. Similarly, nurseries and gardeners would like to be able to reduce the amount of water lost by plants that are to be transplanted, because transplanting often damages the roots, causing the plant to wilt or die. What we need is a good antitranspirant: a compound that can be applied to plants, reducing water loss from the stomata without producing disastrous side effects by excessively limiting carbon dioxide uptake.

Absciscic acid and its commercial chemical analogs have been found to work as antitranspirants in small-scale tests, but their high cost has precluded commercial use. What about making plants more sensitive to their own abscisic acid? The guard cells of plants with a genetic mutation called *era* are highly sensitive to abscisic acid. These plants are resistant to wilting during

drought stress.

A totally different type of antitranspirant seals off the leaves from the atmosphere for a time. Growers use a variety of compounds, most of which form polymeric films around leaves, to form a barrier to evaporation. These compounds cause undesirable side effects, however, and can be used only for relatively short periods of time. Their most common use is in the transplanting of nursery stock.

Crassulacean acid metabolism correlates with an inverted stomatal cycle

Most plants open and close their stomata on a schedule like that shown by the blue curve in Figure 35.11. The stomata are typically open for much of the day and closed at night. (They may also close during very hot days to reduce water loss.) But not all plants follow this pattern.

A brief exposure to blue light against a background of constant, dim red light...

...causes protons to flow out of the cell for a few minutes.

35.7 7 Stomatal Cycles

Most plants open their stomata during the day. CAM plants reverse this stomatal cycle: Their stomata open during the night.

Open

I

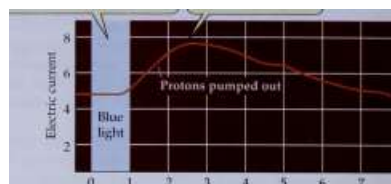
Degree of

stomatal

opening

1

Closed Light/dark periods



3 4 5

Time (minutes)

Many plants that live in dry areas or near the ocean have some unusual biochemical and behavioral features. One particularly surprising feature is their "backward" stomatal cycle: Their stomata are open at night and closed by day (as shown by the red curve in Figure 35.11). This behavior is part of the phenomenon of crassulacean acid metabolism (CAM), which was described in Chapter 8 (see Figure 8.21).

At night, while the stomata are open, carbon dioxide diffuses freely into the leaves of CAM plants and reacts in the mesophyll cells with phosphoenolpyruvic acid to produce organic acids. These acids accumulate to high concentrations. At daybreak the stomata close. Throughout the day, the organic acids are broken down to release the carbon dioxide they contain—behind closed stomata. Because the carbon dioxide cannot diffuse out of the plant, it is available for photosynthesis.

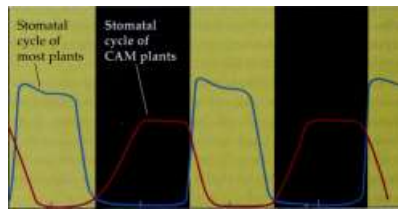
CAM is well adapted to environments where water is scarce: A leaf with its stomata open only at night—when the environment is cooler—loses much less water than does a leaf with its stomata open by day.

In both CAM and non-CAM plants, carbon dioxide is fixed and converted to the products of photosynthesis. How are these products delivered to other parts of the plant?

Translocation of Substances in the Phloem

Substances in the phloem move from sources to sinks. A source is an organ (such as a mature leaf or a storage root) that produces (by photosynthesis or by digestion of stored

Stomatal cycle of most plants



Noon

Midnight

Noon

Midnight

EXPERIMENT

Question: Are organic solutes translocated in the xylem or in the phloem?

METHOD

RESULTS

Remove bark to girdle the tree.

Bark

Wood



Organic solutes accumulate in the phloem above the girdle, causing swelling.

Time



Conclusion: Organic solutes are translocated in the phloem, not in the xylem.

35.12 Girdling Blocks Translocation in the Phloem

By removing a ring of bark (containing the phloem), Malpighi blocked the translocation of organic solutes in a tree.

reserves) more sugars than it requires. A sink is an organ (such as a root, a flower, a developing tuber, or an immature leaf) that does not make enough sugar for its own growth and storage needs. Sugars (primarily sucrose), amino acids, some minerals, and a variety of other substances are translocated between sources and sinks in the phloem.

How do we know that such organic solutes are translocated in the phloem, rather than in the xylem? Just over 300 years ago, the Italian scientist Marcello Malpighi performed a classic experiment in which he removed a ring of bark (containing the phloem) from the trunk of a tree—that is, he girdled the tree (Figure 35.12). The bark in the region above the girdle swelled over time. We now know that the swelling resulted from the accumulation of organic solutes that came from higher up the tree and could no longer continue downward because of the disruption of the phloem. Later,

oo

(/>) Longistigma caryae



Sap droplet

TRANSPORT IN PLANTS 629

the bark below the girdle died because it no longer received sugars from the leaves.

Any model to explain translocation of organic solutes must account for a few important facts:

- ▶ Translocation stops if the phloem tissue is killed by heating or other methods; thus the mechanism must be different from that of transport in the xylem.
- ▶ Translocation often proceeds in both directions—up and down the stem—simultaneously.
- ▶ Translocation is inhibited by compounds that inhibit respiration and thus limit the ATP supply in the source.

To investigate translocation, plant physiologists needed to obtain samples of pure sieve tube sap from individual sieve tube members. This difficult task was simplified when scientists discovered that a common garden pest, the aphid, feeds by drilling into a sieve tube. An aphid inserts its stylet, or feeding organ, into a stem until the stylet enters a sieve tube (Figure 35.13n). Within the sieve tube, the pressure is much greater than in the surrounding plant tissues, so nutritious sieve tube sap is forced up the stylet and into the aphid's digestive tract. So great is the pressure that sugary liquid is forced through the insect's body and out the anus (Figure 35.13b).

Plant physiologists use aphids to collect sieve tube sap. When liquid appears on the aphid's abdomen, indicating that the insect has connected with a sieve tube, the physiologist quickly freezes the aphid and cuts its body away from the stylet, which remains in the sieve tube member. For hours, sieve tube sap continues to exude from the cut stylet, where it may be collected for analysis. Chemical analysis of sieve tube sap collected in this manner reveals the contents of a single sieve tube member over time. We can also infer the rates at which different substances are translocated by measuring how long it takes for radioactive tracers administered to a leaf to appear at stylets at different distances from the leaf.

These methods have allowed us to understand how, at times, different substances might move in opposite directions in the phloem of a stem. Experiments with aphid stylets have shown that all the contents of any given sieve tube member move in the same direction. Thus, bidirectional translocation can be understood in terms of different sieve tubes conducting sap in opposite directions. Data obtained by these and other means led to the general adoption of the pressure flow model as an explanation for translocation in the phloem.

35.13 Aphids Collect Sieve Tube Sap

(a) Aphids feed on phloem sap drawn from the sieve tube, which they penetrate with a modified feeding organ, the stylet, (b) Pressure inside the sieve tube forces sap through the aphid's digestive tract, from which it can be harvested.

630 CHAPTER THIRTY-FIVE

The pressure flow model appears to account for phloem translocation

The tonoplast breaks down during sieve tube member development, allowing the contents of the central vacuole to combine with much of the cytosol to form the sieve tube sap (see Chapter 34). The sap flows under pressure through the sieve tubes. It moves from one sieve tube member to the next by bulk flow through the sieve plates, without crossing a membrane.

Two steps in sieve tube sap flow require metabolic energy:

- ▶ Transport of sucrose and other solutes into the sieve tubes (loading) at sources
- ▶ Removal (unloading) of the solutes where the sieve tubes enter sinks

According to the pressure flow model of translocation in the phloem, sucrose is actively transported into sieve tube members at sources, giving these cells a much greater sucrose concentration than surrounding cells. Water therefore enters the sieve tube members by osmosis. The entry of this water causes a greater pressure potential at the source end, so the entire fluid content of the sieve tube is pushed to-

ward the sink end of the tube—that is, the sap moves by bulk flow (Figure 35.14).

The pressure flow model of translocation in the phloem is contrasted with the transpiration-cohesion-tension model of xylem transport in Table 35.1.

Testing the pressure flow model

The pressure flow model was first proposed more than half a century ago, but some of its features are still debated. Other mechanisms have been proposed to account for translocation in sieve tubes. Some have been disproved, and none of the rest have been supported by a weight of evidence comparable to that for the pressure flow model, which must meet two requirements:

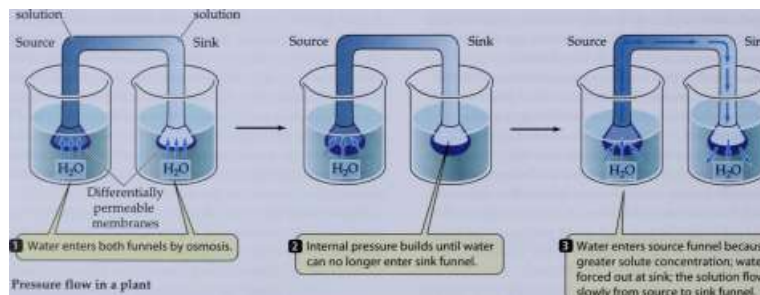
- The sieve plates must be open, so that bulk flow from one sieve tube member to the next is possible.
- There must be an effective method for loading sucrose and other solutes into the phloem in source tissues and removing them in sink tissues.

Let us see whether these requirements are met.

are the sieve plates clogged or open? Early electron microscopic studies of phloem samples cut from plants produced results that seemed to contradict the pressure flow

[a] The pressure flow model More concentrated

Less concentrated



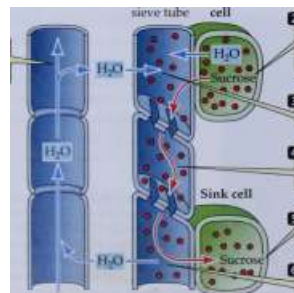
[b] Pressure flow in a plant

Xylem

Q Transpiration pulls water up xylem vessels.

Phloem Source sieve tube cell

o Water enters source funnel because of greater solute concentration; water is forced out at sink; the solution flows slowly from source to sink funnel.



Source cells load sucrose into phloem sieve tubes, reducing their water potential...

...so water is taken up from xylem vessels.

Internal pressure drives the sap down the sieve tube.

Sucrose is unloaded into sink cells...



...and water moves back to xylem vessels.

fc 35. 7 4 The Pressure Flow Model

(a) This demonstration of the pressure flow model shows how pressure potential and water potential combine to drive the bulk flow of sugars and other solutes from a source to a sink, (b) Sap may flow through sieve tubes in this manner.

TRANSPORT IN PLANTS 631

Jj.l Mechanisms of Bulk Flow in Plant Vascular Tissues

XYLEM

PHLOEM

Source of bulk flow Site of bulk flow Pressure potential in sap

Transpiration from leaves

Dead vessel elements and tracheids

Negative (pull from top)

Active transport of sucrose at source Living sieve tube members Positive (push from source)

model. The pores in the sieve plates always appeared to be plugged with masses of a fibrous protein, suggesting that sieve tube sap could not flow freely. But what is the function of that fibrous protein?

One possibility is that this protein is usually distributed more or less at random throughout the sieve tube members until the sieve tube is damaged; then the sudden surge of sap toward the cut surface carries the protein into the pores, blocking them and preventing the loss of valuable nutrients. In other words, perhaps the protein does not block the pores unless the phloem is damaged. How might this possibility be tested? Could we obtain phloem for microscopic observation without causing the sap to surge to the cut surface?

One way to prevent the surge of the sap is to freeze plant tissue before cutting it. Another way is to let the tissue wilt so that there is no pressure in the phloem before cutting. When these methods were used, the sieve plates were not clogged by the protein. Thus, the first condition of the pressure flow model is met.

(a)

Mesophyll cells produce sugars.

Sugars move primarily through the symplast on their way to the sieve tube members.

NEIGHBORING CELLS LOAD AND UNLOAD THE SIEVE TUBE MEMBERS. If the pressure flow model is correct, there must be mechanisms for loading sugars and other solutes into the phloem in source regions and for unloading them in sink regions. One pathway of phloem loading has been demonstrated in some plant species.

Sugars and other solutes pass from cell to cell through the symplast in the mesophyll. When these substances reach cells adjacent to the ends of leaf veins, they leave the mesophyll cells and enter the apoplast, sometimes with the help of transfer cells. Then specific sugars and amino acids are actively transported into cells of the phloem, thus reentering the symplast (Figure 35.15).

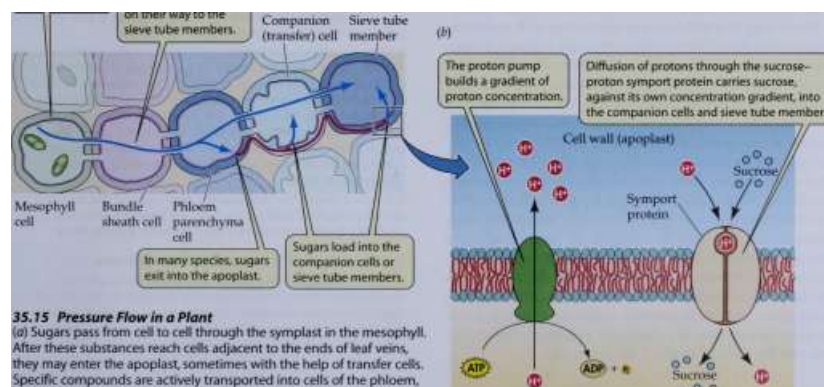
Passage through the apoplast and back into the symplast selects substances to be accumulated for translocation because substances can enter the phloem only after passing through a differentially permeable membrane. In many plants, solutes reenter the symplast at the companion cells (see Chapter 34), which then transfer the solutes to the adjacent sieve tube members. As Figure 35.15 shows, in other plant species, sucrose or other sugars move from the mesophyll to the sieve tube members entirely within the symplast; that is, transfer of solutes from symplast to apoplast and back again is not a universal feature of phloem loading.

Companion (transfer) cell

Sieve tube member

The proton pump builds a gradient of proton concentration.

Diffusion of protons through the sucrose-proton symport protein carries sucrose, against its own concentration gradient, into the companion cells and sieve tube members.



Mesophyll cell

35.15 Pressure Flow in a Plant

(a) Sugars pass from cell to cell through the symplast in the mesophyll. After these substances reach cells adjacent to the ends of leaf veins, they may enter the apoplast, sometimes with the help of transfer cells. Specific compounds are actively transported into cells of the phloem, thus reentering the symplast. (b) Active transport of sugars into the phloem is carried out by sucrose-proton symport, which relies on a proton concentration gradient established by proton pumps.

Cytoplasm of sieve tube member (symplast)

632 CHAPTER THIRTY-FIVE

A form of secondary active transport (see Chapter 5, pages 88-90) loads sucrose into the companion cells and sieve tube members. Sucrose is carried through the plasma membrane from apoplast to symplast by sucrose-proton symport; thus the entry of sucrose and of protons is strictly coupled. For this symport to work, the apoplast must have a high concentration of protons; the protons are supplied by a primary active transport system, the proton pump. The protons then diffuse back into the cell through the symport protein, bringing sucrose with them.

In sink regions, the solutes are actively transported out of the sieve tube members and into the surrounding tissues. This unloading serves two purposes: It helps maintain the gradient of solute potential and hence of pressure potential in the sieve tubes, and it promotes the buildup of sugars and starch to high concentrations in storage regions, such as developing fruits and seeds.

Plasmodesmata and material transfer between cells

Many substances move from cell to cell within the symplast by way of plasmodesmata (see Figure 34.7). Among their other roles, plasmodesmata participate in the loading and unloading of sieve tube members. Mechanisms vary among plant species, but the story in tobacco plants is a common one. In tobacco, sugars and other compounds in source tissues enter companion cells by active transport from the apoplast and move on to the sieve tube members through plasmodesmata. In sink tissues, plasmodesmata connect sieve tube members, companion cells, and the cells that will receive and use the transported compounds.

Plasmodesmata undergo developmental changes as an immature sink leaf matures into a mature source leaf. Plasmodesmata in sink tissues favor rapid unloading: They are more abundant, and they allow the passage of larger molecules. Plasmodesmata in source tissues are few in number.

It was long thought that only substances with molecular weights less than 1,000 could fit through a plasmodesma. Then biologists discovered that cells infected with tobacco mosaic virus (TMV) could allow molecules with molecular weights of as much as 20,000 to exit. We now know that TMV encodes a "movement protein" that produces this change in the permeability of the plasmodesmata—and that plants themselves normally produce at least one such movement protein. Even large molecules such as proteins and RNAs, with molecular weights up to at least 50,000, can thus move between living plant cells. We will see some consequences of this movement of macromolecules through plasmodesmata in later chapters. Biologists are exploring possible ways to regulate the permeability, number, and form of plasmodesmata as a means of modifying traffic in the plant. Such modifications might, for example, allow the diversion of more of a grain crop's photosynthetic products into the grain, increasing the crop yield.

Chapter Summary

Uptake and Transport of Water and Minerals

- ▶ Plant roots take up water and minerals from the soil.
- ▶ Water moves through biological membranes by osmosis, always moving toward cells with a more negative water potential. The water potential of a cell or solution is the sum of the solute potential and the pressure potential. All three parameters are expressed in megapascals (MPa). Review Figure 35.1
- ▶ Mineral uptake requires transport proteins. Some minerals enter the plant by facilitated diffusion; others enter by active transport. A proton pump facilitates the active transport of many solutes across membranes in plants. Review Figure 35.2
- ▶ Water and minerals pass from the soil to the xylem by way of the apoplast and symplast. In the root, water and minerals may pass from the cortex into the stele only by way of the symplast because Casparian strips in the endodermis block water and solute movement in the apoplast. Review Figures 35.3, 35.4

Transport of Water and Minerals in the Xylem

- ▶ Early experiments established that sap does not move via the pumping action of living cells.
- ▶ Root pressure is responsible for guttation and for the oozing of sap from cut stumps, but it cannot account for the ascent of sap in trees.
- ▶ Xylem transport is the result of the combined effects of transpiration, cohesion, and tension. Evaporation in the leaf produces tension in the surface film of water on the moist-walled mesophyll cells, and thus pulls water—held together by its cohesiveness—up through the xylem from the root. Dissolved minerals go along for the ride. Review Figure 35.7
- ▶ Support for the transpiration-cohesion-tension model of xylem transport came from studies using a pressure bomb.

Review Figure 35.8

Transpiration and the Stomata

- ▶ Evaporation of water cools the leaves, but a plant cannot afford to lose too much water. Transpirational water loss is minimized by the waxy cuticle of the leaves.
- ▶ Stomata allow a compromise between water retention and carbon dioxide uptake. A pair of guard cells controls the size of the stomatal opening. A proton pump, activated by blue light, pumps protons from the guard cells to surrounding epidermal cells. As a result, the guard cells take up potassium ions, causing water to follow osmotically, swelling the cells and opening the stomata. Carbon dioxide level and water availability also affect stomatal opening. Review Figures 35.9, 35.10
- ▶ In most plants the stomata are open during the day and closed at night. CAM plants have an inverted stomatal cycle, enabling them to conserve water. Review Figure 35.11

Translocation of Substances in the Phloem

- ▶ Products of photosynthesis, and some minerals, are translocated through sieve tubes in the phloem by way of living sieve tube members. Translocation proceeds in both directions in the stem, although in a single sieve tube it goes only one way. Translocation requires a supply of ATP.

TRANSPORT IN PLANTS 633

- ▶ Translocation in the phloem proceeds in accordance with the pressure flow model: The difference in solute concentration between sources and sinks allows a difference in pressure potential along the sieve tubes, resulting in bulk flow. Review Figure 35.14, Table 35.1
- ▶ The pressure flow model succeeds because the sieve plates are normally open, allowing bulk flow, and because neighboring cells load organic solutes into the sieve tube members in source regions and unload them in sink regions. Review Figure 35.15
- ▶ The distribution and properties of plasmodesmata differ between source and sink tissues. It may become possible to regulate plasmodesmata in crop plants.

For Discussion

1. Epidermal cells protect against excess water loss. How do they perform this function?
2. Phloem transports material from sources to sinks. What is meant by "source" and "sink"? Give examples of each.
3. What is the minimum number of plasma membranes a water molecule would have to cross in order to get from the soil solution to the atmosphere by way of the stele? To get from the soil solution to a mesophyll cell in a leaf.
4. Transpiration exerts a powerful pulling force on the water column in the xylem. When would you expect transpiration to proceed most rapidly? Why? Describe the source of the pulling force.
5. Plants that perform crassulacean acid metabolism (CAM plants) are adapted to environments in which water supply is limited; these plants open their stomata only at night. Could a non-CAM plant, such as a pea plant, enjoy an advantage if it opened its stomata only at night? Explain.

36

Plant Nutrition



An insect has stepped on a trigger hair on the leaf of a Venus flytrap—a big mistake for the insect. The trigger hair sends an electrical signal that springs a mechanical trap. The two halves of the leaves close, and spiny outgrowths at the margins of the leaves interlock to imprison the insect. The leaf secretes enzymes that will digest its prey. The leaf then absorbs the products of digestion, especially amino acids, and uses them as a nutritional supplement.

Why does the Venus flytrap go to all this trouble? Few other plants are carnivorous—your petunia plant is not stalking you, after all. But the Venus flytrap (*Dionaea muscipula*) lives on soils in which nitrogen is scarce. Its carnivorous adaptation gives it another way to obtain needed nitrogen.

Why do plants need nitrogen? The answer is simple if we recall the chemical structures of proteins and nucleic acids that we looked at in Chapter 3. These vital components of all living things contain nitrogen, as do chlorophyll and many other important biochemical compounds. If a plant cannot get enough nitrogen, it cannot synthesize these compounds at a rate adequate to keep itself healthy.

In addition to nitrogen, plants need other materials from their environment. In this chapter we explore the differences

between the basic strategies of plants and animals for obtaining nutrition. Then we look at what nutrients plants require, and how they acquire them. Because most nutrients come from the soil, we discuss the formation of soils and the effects of plants on soils. As any farmer can tell you, nitrogen is the nutrient that most often limits plant growth, so we devote a section specifically to nitrogen metabolism in plants. The chapter concludes with a look at plants that use means other than photosynthesis to supplement their nutrition.

The Acquisition of Nutrients

Every living thing must obtain raw materials from its environment. These nutrients include the major ingredients of macromolecules: carbon, hy-

A Meat-Eating Plant

Dionaea muscipula, the Venus flytrap, has adapted to a nitrogen-poor environment by becoming carnivorous. It obtains this necessary mineral from the bodies of insects trapped inside the plant when its hinges snap shut.

drogen, oxygen, and nitrogen. Carbon and oxygen enter the living world through the carbon-fixing reactions of photosynthesis, in which photosynthetic organisms obtain them from atmospheric carbon dioxide. Hydrogen enters living systems through the light reactions of photosynthesis, which split water. For carbon, oxygen, and hydrogen, photosynthesis is the gateway to the living world.

The movement of nitrogen into organisms begins with processing by some highly specialized bacteria living in the soil. Some of these bacteria act on nitrogen gas, converting it into a form usable by plants. The plants in turn provide organic nitrogen and carbon to animals, fungi, and many microorganisms.

In addition to carbon, oxygen, hydrogen, and nitrogen, other mineral nutrients are essential to living systems. The proteins of organisms contain sulfur (S), and their nucleic acids contain phosphorus (P). There is magnesium (Mg) in chlorophyll, and iron (Fe) in many important compounds, such as the cytochromes. Within the soil, these and other minerals dissolve in water, forming a solution—called the soil solution—that contacts the roots of plants. Plants take up most of these mineral nutrients from the soil solution in ionic form.



Autotrophs make their own organic compounds

The plants provide carbon, oxygen, hydrogen, nitrogen, and sulfur to most of the rest of the living world. Plants, some protists, and some bacteria are autotrophs; that is, they make their own organic compounds from simple inorganic nutrients—carbon dioxide, water, nitrate or ammonium ions containing nitrogen, and a few other soluble mineral nutrients (Figure 36.1). Heterotrophs are organisms that require preformed organic compounds (compounds that contain carbon) as food. Herbivores and carnivores are heterotrophs that depend directly or indirectly on autotrophs as their source of nutrition.

Most autotrophs are photosynthesizers—that is, they use light as the source of energy for synthesizing organic compounds from inorganic raw materials. Some autotrophs, however, are chemosynthesizers, deriving their energy not from light, but from reduced inorganic substances, such as hydrogen sulfide (H_2S), in their environment. All chemosynthesizers are bacteria. Some chemosynthetic bacteria in the soil contribute to the nutrition of plants by increasing the availability of nitrogen and sulfur. But how does a plant obtain its nutrients, whether they come with or without bacterial action?

How does a stationary organism find nutrients?

An organism that cannot move must exploit energy that is somehow brought to it. Most sessile animals depend primarily on the movement of water to bring energy, in the form of food, to them, but a plant's supply of energy arrives at the speed of light from the sun. A plant's supply of nutrients, however, is strictly local, and the plant may use up the water and mineral nutrients in its local environment as it develops. How does a plant cope with such a problem?

One answer is to extend itself by growing into new re-sources. Growth is a plant's version of locomotion. Root systems mine the soil; by growing, they reach new sources of mineral nutrients and water. Growth of leaves helps a plant se-



cure light and carbon dioxide. A plant may compete with other plants for light by outgrowing them, both capturing more light for itself and preventing the growth of its neighbors by shading them.

As it grows, a plant—or even a single root—must deal with environmental heterogeneity. Animal droppings create high local concentrations of nitrogen. A particle of calcium carbonate in the soil may make a tiny area alkaline, while dead organic matter may make a nearby area acidic.

Mineral Nutrients Essential to Plants

What important mineral nutrients do plants take up from their environment, and what are their roles? Table 36.1 lists the mineral nutrients that have been proved to be essential for plants. Except for nitrogen, they all come from the soil solution and derive ultimately from rock.

There are three criteria for calling something an essential element:

- ▶ The element must be necessary for normal growth and reproduction.
- ▶ The element cannot be replaceable by another element.
- ▶ The requirement must be direct —that is, not the result of an indirect effect, such as the need to relieve toxicity caused by another substance.

In this section, we'll consider the symptoms of particular mineral deficiencies, the roles of some of the mineral nutrients, and the technique by which the essential elements for plants were identified.

The essential elements in Table 36.1 are divided into two categories: the macronutrients and the micronutrients. Plant tissues need macronutrients in concentrations of at least 1 gram per kilogram of their dry matter, and they need micronutrients in concentrations of less than 100 milligrams per kilogram of their dry matter. (Dry matter, or dry weight, is what remains after all the water has been removed from a tissue sample.) Both the macronutrients and the micronutrients are essential for the plant to complete its life cycle from seed to seed.

Deficiency symptoms reveal inadequate nutrition

Before a plant that is deficient in an essential element dies, it usually displays characteristic deficiency symptoms. Table 36.2 describes the symptoms of some common mineral deficiencies. Such symptoms help horticulturists diagnose mineral nutrient deficiencies in plants.

36.7 What Do Plants Need?

To survive, plants require only light plus carbon dioxide, water, and several essential mineral elements. These plants are growing on nothing more than a solution that contains water and mineral elements. This technique is known as hydroponic culture.

636 CHAPTER THIRTY-SIX

7 O. / Mineral Elements Required by Plants

ELEMENT

ABSORBED FORM

MAJOR FUNCTIONS

In proteins, nucleic acids, etc.

In nucleic acids, ATP, phospholipids, etc.

Enzyme activation; water balance; ion balance; stomatal opening

In proteins and coenzymes

Affects the cytoskeleton, membranes, and many enzymes; second messenger

In chlorophyll; required by many enzymes; stabilizes ribosomes

In active site of many redox enzymes and electron carriers; chlorophyll synthesis

Photosynthesis; ion balance

Activation of many enzymes

Possibly carbohydrate transport (poorly understood)

Enzyme activation; auxin synthesis

In active site of many redox enzymes and electron carriers

Activation of one enzyme

Nitrogen fixation; nitrate reduction

Nitrogen deficiency is the most common mineral deficiency in plants. Plants in natural environments are almost always deficient in nitrogen, but they seldom display deficiency symptoms. Instead, their growth slows to match the available supply of nitrogen. Crop plants, on the other hand, show deficiency symptoms if a formerly abundant supply of nitrogen runs out. The visible symptoms of nitrogen deficiency include uniform yellowing, or chlorosis, of older leaves. Chlorophyll, which is responsible for the green color of leaves, contains nitrogen. Without nitrogen there is no chlorophyll, and without chlorophyll, the yellow pigments become visible.

Zinc

yellow veins

Young leaves are abnormally small; older leaves have many dead spots

Inadequate available iron in the soil can also cause chlorosis because, although it is not contained in the chlorophyll molecule, iron is required for chlorophyll synthesis. However, iron deficiency commonly causes chlorosis of the youngest leaves, with their veins sometimes remaining green. The reason for this difference is that nitrogen is readily translocated in the plant and can be redistributed from older tissues to younger tissues to favor their growth. Iron, on the other hand, cannot be readily redistributed. Younger tissues that are actively growing and synthesizing compounds needed for their growth show iron deficiency before older leaves, which have already completed their growth.

Several essential elements fulfill multiple roles

Essential elements can play several different roles—some structural, others catalytic. Magnesium, as we have mentioned, is a constituent of the chlorophyll molecule and hence is essential to photosynthesis. It is also required as a cofactor by numerous enzymes in cellular respiration and other metabolic pathways.

Phosphorus, usually in phosphate groups, is found in many organic compounds, particularly in nucleic acids and in the intermediates of the energy pathways of photosynthesis and glycolysis. The transfer of phosphate groups occurs in many energy-storing and energy-releasing reactions, notably those that use or produce ATP. Other roles of phosphate groups include the activation and inactivation of enzymes.

Calcium plays many roles in plants. Its function in the processing of hormonal and environmental cues is the subject of great biological interest, as we'll see in the next chapter. Calcium also affects membranes and cytoskeleton activity, participates in spindle formation for mitosis and meiosis, and is a constituent of the middle lamella of cell

EXPERIMENT

Question: Is a particular ingredient of a growth medium an essential plant nutrient?

METHOD Grow seedlings in a medium that lacks the element in question (in this case, nitrogen)

Control



Experiment

Seedling grown in a complete growth medium.

RESULTS



Seedling grown in a medium lacking nitrogen.



Conclusion: Nitrogen is an essential plant nutrient



n



36.2 Identifying Essential Elements for Plants

The diagram shows the procedure for identifying nutrients essential to plants, using nitrogen as an example. The environment in such experiments must be rigorously controlled because some essential elements are needed in only tiny amounts, and may be present in sufficient quantities as contaminants.

walls. Other elements, such as iron and potassium, also play multiple roles.

All of these elements are essential to the life of all plants. How did biologists discover which elements are essential?

The identification of essential elements

An element is considered essential if a plant fails to complete its life cycle, or grows abnormally, when that element is not available, or is not available in sufficient quantities. Plant physiologists identified most of the essential elements for plants by the technique outlined in Figure 36.2. This technique is limited, however, by the possibility that some elements thought to be absent from the test solutions are actually present. Impurities and contamination are always possible.

In early experiments on plant nutrition, some of the chemicals used were so

36.3 The Complexity of Soil

Soil has both organic and inorganic components.

PLANT NUTRITION 637

impure that they provided micronutrients that the investigators thought they had excluded. Some mineral nutrients are required in such tiny amounts that there may be enough in a seed to supply the embryo and the resultant plant throughout its lifetime and leave enough in the next seed to get the next generation well started. Simply touching a plant may give it a significant dose of chlorine in the form of chloride ions from sweat. Only rarely are new essential elements reported now. Either the list is nearly complete, or more likely, we will need more sophisticated techniques to add to it.

Where does the plant find its essential mineral nutrients? How does it absorb them?

Soils and Plants

Soils are very important to plants, and plant interactions with the soil are complex. Plants obtain their mineral nutrients from the soil solution or the water in which they grow. Water for terrestrial plants also comes from the soil, as does the supply of oxygen for the roots. Soil also provides mechanical support for plants on land, and it harbors bacteria that perform chemical reactions leading to products required for plant growth. On the other hand, soil may also contain organisms harmful to plants.

In the pages that follow, we'll examine the composition and structure of soils, their formation, their role in plant nutrition, their care and supplementation in agriculture, and their modification by the plants that grow in them.

Soils are complex in structure

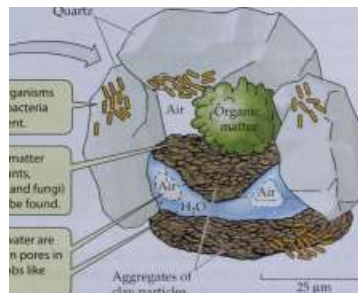
Soils are complex systems of living and nonliving components. The living components include plant roots, as well as populations of bacteria, fungi, and animals such as earthworms and insects (Figure 36.3). The nonliving portion of the soil includes rock fragments ranging in size from large



Soil consists of more than inorganic mineral particles such as clay and quartz.

Quartz

Living organisms such as bacteria are present.



Organic matter (from plants, animals, and fungi) can also be found.

Air and water are present in pores in soil crumbs like this one.

Aggregates of clay particles

638 CHAPTER THIRTY-SIX

boulders to tiny particles called clay that are 2 μm or less in diameter (Table 36.3). Soils also contain water and dissolved mineral nutrients, air spaces, and dead organic matter. The air spaces are crucial sources of oxygen for plant roots. Soils change constantly through natural causes—such as rain, temperature extremes, and the activities of plants and animals—as well as human activities—farming in particular.

The structure of many soils changes with depth, revealing a soil profile. Although soils differ greatly, almost all soils consist of two or more horizons—recognizable horizontal layers—lying on top of one another. Mineral nutrients tend to be leached—dissolved in rain or irrigation water and carried to deeper horizons.

Soil scientists recognize three major zones (A, B, and C) in the profile of a typical soil (Figure 36.4). Topsoil is the A horizon, from which mineral nutrients may be depleted by leaching. Most of the organic matter in the soil is in the

C horizon Weathering parent rock

(bedrock)



36.4 A Soil's Profile

The A, B, and C horizons can sometimes be seen in road cuts such as this one in Australia. The dark upper layer (A horizon) is home to most of the living organisms in the soil.

A horizon, as are most roots, earthworms, insects, nematodes, and microorganisms. Successful agriculture depends on the

presence of a suitable A horizon. Pure sand contains plenty of air spaces, but is low in water and mineral nutrients. Clay contains lots of nutrients and more water than sand does, but it is low in air. A little bit of clay goes a long way in affecting soil properties. A loam has significant amounts of sand, silt, and clay, and thus has good levels of air, water, and nutrients for plants. Most of the best topsoils for agriculture are loams.

Below the A horizon is the B horizon, or subsoil, which is the zone of infiltration and accumulation of materials leached from above. Farther down, the C horizon is the original parent rock from which the soil is derived. Some deep-growing roots extend into the B horizon, but roots rarely enter the C horizon.

Soils form through the weathering of rock

The type of soil in a given area depends on the type of rock from which it formed, the climate, the landscape features, the organisms living there, and the length of time that soil-forming processes have been acting (sometimes millions of years). Rocks are broken down in part by mechanical weathering, which is the physical breakdown—without any accompanying chemical changes—of materials by wetting, drying, and freezing. The most important parts of soil formation, however, include chemical weathering, the chemical alteration of at least some of the materials in the rocks.

The key process is the formation of clay. Both the physical and the chemical properties of soils depend on the amount and kind of clay particles they contain. Just grinding up rocks does not produce a clay that binds mineral nutrients and aggregates into particles. Such a clay results only from chemical weathering.

Soils are the source of plant nutrition

The supply of mineral nutrients for plants depends on the presence of clay particles in the soil. Many of the minerals that are important for plant nutrition, such as potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}), exist in soil as positively charged ions, or cations. Clay particles have a net negative charge, which they get from negatively charged ions that are permanently attached to them. Cations in solution are attracted to these negative ions. To become available to plants, the cations must be detached from the clay particles.

This task is accomplished by reactions with protons (hydrogen ions, H^+). Roots release protons into the soil, and they are also released by the ionization of carbonic acid (H_2CO_3), which is formed whenever CO_2 from respiring roots or from the atmosphere dissolves in water. Protons bond more strongly to the clay particles than do the mineral cations, so they trade places with the cations, thus putting the nutrients back into the soil solution. This trading of places is called ion exchange (Figure 36.5). The fertility of a soil is determined in part by its ability to provide nutrients in this manner.

Clay particles effectively hold and exchange positively charged ions, but there is no comparable mechanism for exchanging negatively charged ions. As a result, important negative ions such as nitrate (NO_3^-) and sulfate (SO_4^{2-})—the primary and direct sources of nitrogen and sulfur—leach rapidly from soil, whereas positive ions tend to be retained in the A horizon. The reservoir of soil nitrogen is the organic matter in the soil, which slowly decomposes to release nitrogen in a form available to plants.

Fertilizers and lime are used in agriculture

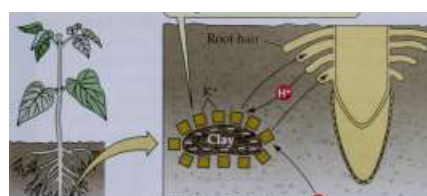
Agricultural soils often require fertilizers because irrigation and rainwater leach mineral nutrients from the soil, and the harvesting of crops removes the nutrients that the plants took up from the soil during their growth. Crop yields decrease if any essential element is depleted. Mineral nutrients may be replaced by organic fertilizers, such as rotted manure, or inorganic fertilizers of various types. The three elements most commonly added to agricultural soils are nitrogen (N), phosphorus (P), and potassium (K). Commercial fertilizers are characterized by their "N-P-K" percentages. A 5-10-10 fertilizer, for example, contains 5 percent nitrogen, 10 percent phosphate (P_2O_5), and 10 percent potash (K_2O) by weight.* Sulfur, in the form of a sulfate, is also occasionally added to soils.

Either organic or inorganic fertilizers can provide the necessary mineral nutrients for plants. Organic fertilizers release nutrients slowly, which results in less leaching than a one-time application of inorganic fertilizer. Organic fertilizers also contain materials that improve the physical properties of the soil, providing spaces for gas movement, root growth, and drainage. Inorganic fertilizers, on the other hand, provide an almost instantaneous supply of soil nutrients and can be formulated to meet the requirements of a particular soil and a particular crop.

The availability of nutrient ions, whether they are naturally present in the soil or added as fertilizer, is altered by changes in soil pH. The optimal soil pH for most crops is about 6.5, but so-called acid-loving crops such as blueberries prefer a pH closer to 4. Rainfall and the decomposition of

*The analysis is by weight and is not reported as elemental N, P, and K. A 5-10-10 fertilizer actually does contain 5 percent nitrogen, but only 4.3 percent phosphorus and 8.3 percent potassium on an elemental basis.

A clay particle, which is negatively charged, binds cations.





The cations are exchanged for hydrogen ions obtained from carbonic acid (H_2CO_3) or from the plant itself.

36.5 Ion Exchange

Plants obtain mineral nutrients from the soil primarily in the form of positive ions; potassium is the example shown here.

organic substances in the soil lower its pH. Such acidification of the soil can be reversed by liming—the application of compounds commonly known as lime, such as calcium carbonate, calcium hydroxide, or magnesium carbonate. The addition of these compounds leads to the removal of H^+ ions from the soil. Liming also increases the availability of calcium to plants, which require it as a macronutrient.

It is easy to guess how humans learned to use fertilizer: It didn't take much insight to notice improved plant growth around animal feces. Perhaps a similar observation of limestone, or chalk, or oyster shells—all sources of calcium carbonate—led to the practice of liming. Sometimes, on the other hand, a soil is not acidic enough. In this case, a farmer can add sulfur, and soil bacteria will convert it to sulfuric acid. Iron and some other elements are more available to plants at a slightly acidic pH. Soil pH testing is useful for home gardens and lawns as well as for agriculture.

Spraying leaves with a nutrient solution is another effective way to deliver some essential elements to growing plants. Plants take up more copper, iron, and manganese when these elements are applied as foliar (leaf) sprays than when they are added to the soil as fertilizer. Adjusting the concentrations of nutrient ions and pH in order to optimize uptake and to minimize toxicity can yield excellent results. Such foliar application of mineral nutrients is increasingly used in wheat production, but fertilizer is still delivered most commonly by way of the soil.

Plants affect soils

The soil that forms in a particular place also depends on the types of plants growing there. Plant litter, such as dead fallen leaves, is the major source of carbon-rich materials that break down to form humus—dark-colored organic material, each particle of which is too small to be recognizable with the naked eye. Soil bacteria and fungi produce

640 CHAPTER THIRTY-SIX

humus by breaking down plant litter, animal feces, and other organic material. Humus is rich in mineral nutrients, especially nitrogen. Humus in combination with clay promotes a soil structure favorable to plant growth, promoting adequate supplies of both water and oxygen to the roots.

Plants affect the pH of the soil in which they grow. Roots maintain a balance of electric charges. If they absorb more cations than anions, they excrete H^+ ions, thus lowering the soil pH. If they absorb more anions than cations, they excrete OH^- ions or HCO_3^- ions, raising the soil pH.

The mineral nutrient most commonly limiting, in both natural and agricultural situations, is nitrogen. Let's consider how nitrogen is made available to plants.

Nitrogen Fixation

Earth's atmosphere is a vast reservoir of nitrogen in the form of nitrogen gas (N_2). N_2 constitutes almost four-fifths of the atmosphere. However, plants cannot use N_2 directly as a nutrient. It is a highly unreactive substance—the triple bond linking the two nitrogen atoms is extremely stable, and a great deal of energy is required to break it.

A few species of bacteria have an enzyme that enables them to convert N_2 into a more reactive form by a process called nitrogen fixation. These prokaryotic organisms—nitrogen fixers—convert N_2 to ammonia (NH_3). There are relatively few species of nitrogen fixers, and their biomass is small relative to the mass of other organisms that depend on them for survival on Earth. This talented group of prokaryotes is just as essential to the biosphere as are the photosynthetic autotrophs.

Nitrogen fixers make all other life possible

By far the greatest share of total world nitrogen fixation is performed biologically by nitrogen-fixing organisms, which fix approximately 170 million Mg (megagrams, metric tons) of nitrogen per year. About 80 million Mg is fixed industrially by humans. A smaller amount of nitrogen is fixed in the atmosphere by nonbiological means such as lightning, volcanic eruption, and forest fires. Rain brings these atmospherically formed products to the ground.

Several groups of bacteria fix nitrogen. In the oceans, various photosynthetic bacteria, including cyanobacteria, fix nitrogen. In fresh water, cyanobacteria are the principal nitrogen fixers. On land, free-living soil bacteria make some contribution to nitrogen fixation, but they fix only what they need for their own use and release the fixed nitrogen only when they die. Other nitrogen-fixing bacteria live in close association with plant roots. They release up to 90 percent of the nitrogen they fix to the plant and excrete some amino acids into the soil, making nitrogen immediately available to other organisms.

Bacteria of the genus *Rhizobium* fix nitrogen only in close association with the roots of plants in the legume family (Figure 36.6). The legumes include peas, soybeans, clover, alfalfa, and many tropical shrubs and trees. The bacteria infect the plant's roots, and the roots develop nodules in re-



36.6 Root Nodules

Large, round, tumorlike nodules are visible in the root system of a broad bean. These nodules house nitrogen-fixing bacteria.

Response to their presence. The various species of *Rhizobium* show a fairly high specificity for the species of legume they infect. Farmers and gardeners coat legume seeds with *Rhizobium* to make sure the bacteria are present. Some farmers alternate their crops, planting clover or alfalfa occasionally to increase the available nitrogen content of the soil.

The legume-*Rhizobium* association is not the only bacterial association that fixes nitrogen. Some cyanobacteria fix nitrogen in association with fungi in lichens or with ferns, cycads, or nontracheophytes. Rice farmers can increase crop yields by growing the water fern *Azolla*, with its symbiotic nitrogen-fixing cyanobacterium, in the flooded fields where rice is grown. Another group of bacteria, the filamentous actinomycetes, fix nitrogen in association with root nodules on woody species such as alder and mountain lilacs.

How does biological nitrogen fixation work? In the four sections that follow, we'll consider the role of the enzyme nitrogenase, the mutualistic collaboration of plant and bacterial cells in root nodules, the need to supplement biological nitrogen fixation in agriculture, and the contributions of plants and bacteria to the global nitrogen cycle.

Nitrogenase catalyzes nitrogen fixation

Nitrogen fixation is the reduction of nitrogen gas. It proceeds by the stepwise addition of three pairs of hydrogen atoms (Figure 36.7). In addition to N_2 , these reactions require a strong reducing agent to transfer hydrogen atoms to nitrogen and the intermediate products, as well as a great deal of energy, which is supplied by ATP. Depending on the species of nitrogen fixer, either respiration or photosynthesis

Q Under anaerobic conditions, the enzyme nitrogenase binds a molecule of nitrogen gas.



Substrate: Nitrogen gas, N_2

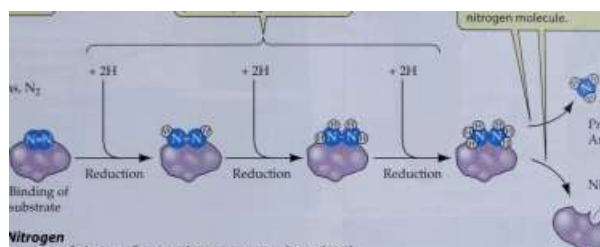
^

^|The nitrogen is reduced by the addition of three successive pairs of hydrogen atoms.

Nitrogenase

Binding of substrate

| The final products—two molecules of ammonia—are released, freeing the nitrogenase to bind another nitrogen molecule.



Product: Ammonia, NH_3

Nitrogenase

36.7 Nitrogenase Fixes Nitrogen

Throughout the chemical reactions of nitrogen fixation, the reactants are bound to the enzyme nitrogenase. A reducing agent

transfers hydrogen atoms to nitrogen, and eventually the final product—ammonia—is released.

sis may provide both the necessary reducing agent and ATP. The reactants are firmly bound to the surface of a single enzyme, called nitrogenase.

Nitrogenase is so strongly inhibited by oxygen (O_2) that its discovery was delayed because investigators had not thought to seek it under anaerobic conditions. Because nitrogenase cannot function in the presence of oxygen, it is not surprising that many nitrogen fixers are anaerobes. Legumes respire aerobically, as do *Rhizobium*. Within a root nodule, oxygen is maintained at a level sufficient to support respiration but not so high as to inactivate nitrogenase.

Some plants and bacteria work together to fix nitrogen

The legume nodule provides an excellent example of symbiosis, in which two different organisms live in physical contact. In the form of symbiosis called mutualism, both organisms benefit from their relationship. The legume obtains fixed nitrogen from the bacterium, and the bacterium obtains the products of photosynthesis from the plant. Neither free-living *Rhizobium* species nor uninfected legumes can fix nitrogen. Only when the two are closely associated in root nodules does the reaction take place.

The establishment of this symbiosis between *Rhizobium* and a legume requires a complex series of steps, with active contributions by both the bacteria and the plant root (Figure 36.8). First the root releases flavonoids and other chemical signals that attract the *Rhizobium* to the vicinity of the root. Flavonoids trigger the transcription of bacterial nod genes, which encode Nod (nodulation) factors. These factors, secreted by the bacteria, cause cell divisions in the root cortex, leading to the formation of a primary nodule meristem. Within the nodules, the bacteria take the form of bacteroids within membranous vesicles. Bacteroids are swollen, deformed bacteria that can fix nitrogen.

Before the bacteroids can begin to fix nitrogen, the plant must produce the protein leghemoglobin, which sur-

rounds the bacteroids. Leghemoglobin is a close relative of hemoglobin, the oxygen-carrying pigment of animals. Some plant nodules contain enough of it to be bright pink when viewed in cross section. Leghemoglobin, with its iron-containing heme, transports oxygen to the bacteroids to support their respiration.

The partnership between bacterium and plant in nitrogen-fixing nodules is not the only case in which plants depend on other organisms for assistance with their nutrition. Another example that we considered earlier is that of mycorrhizae, root-fungus associations in which the fungus greatly increases the absorption of water and minerals (especially phosphorus) by the plant (see Figure 30.16). A growing body of evidence suggests that nodule formation depends on some of the same genes and mechanisms that allow mycorrhizae to develop.

Biological nitrogen fixation does not always meet agricultural needs

Bacterial nitrogen fixation is not sufficient to support the needs of agriculture. Traditional farmers used to plant dead fish along with corn so that the decaying fish would release fixed nitrogen that the developing corn could use. Industrial nitrogen fixation is becoming ever more important to world agriculture because of the degradation of soils and the need to feed a rapidly expanding population. Research on biological nitrogen fixation is being vigorously pursued, with commercial applications very much in mind.

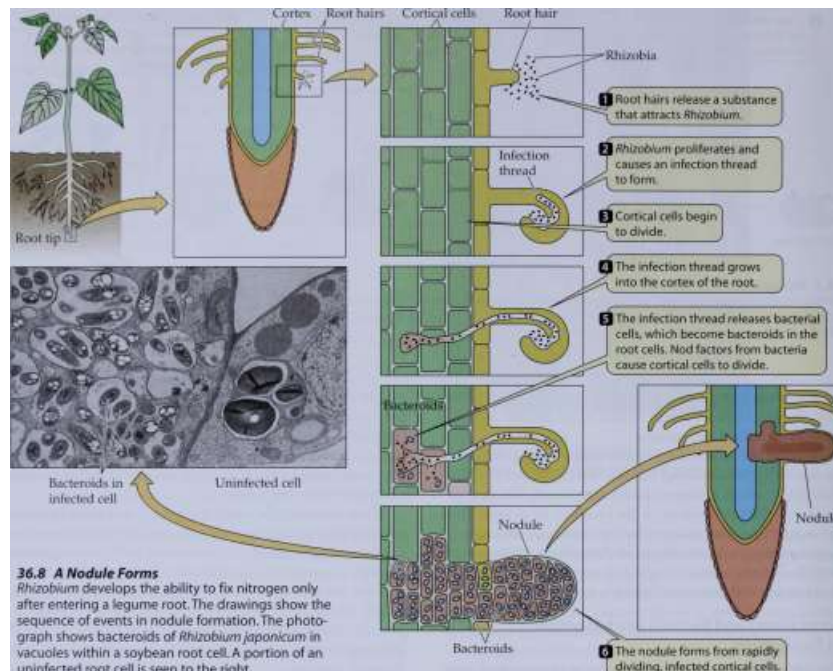
Most industrial nitrogen fixation is done by a chemical process called the Haber process, which requires a great deal of energy. An alternative is urgently needed because of the cost of energy. At present, the manufacture of nitrogen-containing fertilizer takes more energy than does any other aspect of crop production in the United States.

One line of investigation centers on recombinant DNA technology as a means of engineering new plants that produce their own nitrogenase. Workers in many industrial

642 CHAPTER THIRTY-SIX

Root hairs Cortical cells

Root hair



Bacteroids in infected cell

36.8 A Nodule Forms

Rhizobium develops the ability to fix nitrogen only after entering a legume root. The drawings show the sequence of events in nodule formation. The photograph shows bacteroids of *Rhizobium japonicum* in vacuoles within a soybean root cell. A portion of an uninfected root cell is seen to the right.

The nodule forms from rapidly dividing, infected cortical cells.

and academic laboratories are attempting to insert bacterial genes coding for nitrogenase into the cells of angiosperms, particularly crop plants. Developing crops that can fix their own nitrogen, however, will take more than just the insertion of genes for nitrogenase. Biotechnology must also find ways to exclude O_2 and obtain strong reducing agents and an energy source. Ultimately, the need for ATP represents a greater technical challenge than the insertion of nitrogenase genes. The stakes, however—especially the financial ones—are high, and a huge amount of effort is being invested in research along these lines.

Plants and bacteria participate in the global nitrogen cycle

The reduced nitrogen released into the soil by nitrogen fixers is primarily in the form of ammonia (NH_3) and ammonium ions (NH_4^+). Although ammonia is toxic to plants, ammonium ions can be taken up safely at low concentrations. Soil bacteria called nitrifiers, which we described in chapter 26, oxidize ammonia to nitrate ions (NO_3^-)—an-

other form that plants can take up—by the process of nitrification. Soil pH affects uptake: Nitrate ions are taken up preferentially under more acidic conditions, ammonium ions under more basic ones.

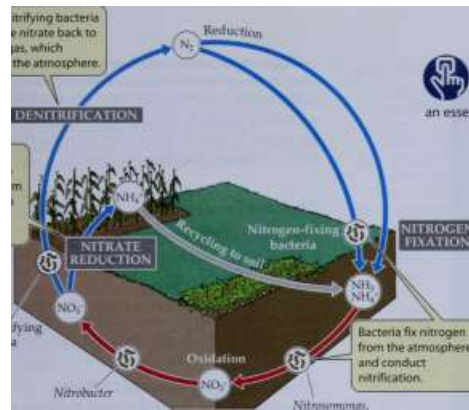
The steps that we have followed so far are carried out by bacteria: N_2 is reduced to ammonia in nitrogen fixation and ammonia is oxidized to nitrate in nitrification. The next steps are carried out by plants, which reduce the nitrate they have taken up all the way back to ammonia (Figure 36.9). All the reactions of nitrate reduction are carried on by the plant's own enzymes. The later steps, from nitrite (NO_2^-) to ammonia, take place in the chloroplasts, but this conversion is not part of photosynthesis. The plant uses the ammonia thus formed to manufacture amino acids, from which the plant's proteins and all its other nitrogen-containing compounds are formed. Animals cannot reduce nitrogen, and they depend on plants to supply them with reduced nitrogenous compounds.

Bacteria called denitrifiers return nitrogen from animal wastes and dead organisms to the atmosphere as N_2 . This process, described in Chapter 26, is called denitrification.

Some denitrifying bacteria can reduce nitrate back to nitrogen gas, which returns to the atmosphere.

Plants reduce nitrates back into ammonia, the form in which nitrogen is incorporated into proteins.

Denitrifying bacteria



Nitrobacter

HiWaWftiWI

Nitrosomonas, Nitrosococcus

In combination with leaching and the removal of crops, it keeps the level of available nitrogen in soils low.

Nitrogen metabolism, in bacteria and in plants, is complex. It is also of great importance: Nitrogen-containing compounds constitute 5 to 30 percent of a plant's total dry weight. The nitrogen content of animals is even higher, and all the nitrogen in the animal world arrives there by way of the plant kingdom.

Sulfur Metabolism

All living things require sulfur, which is a constituent of two amino acids, cysteine and methionine, and hence of almost all proteins. Sulfur is also a component of other biologically crucial compounds, such as coenzyme A. Animals must obtain their cysteine and methionine from plants, but plants can make their own, using sulfate ions obtained from the soil or from a liquid environment.

Except for oxygen, all of the most abundant elements in plants are taken up from the environment in their most oxidized forms—sulfur as sulfate, carbon as carbon dioxide, nitrogen as nitrate, phosphorus as phosphate, and hydrogen as water. In plants, sulfate is reduced and incorporated into cysteine. From this amino acid all the other sulfur-containing compounds in the plant are made. Sulfate reduction and the utilization of cysteine are analogous to the reduction of nitrate to ammonia and the subsequent utilization of ammonia by plants.

Heterotrophic and Carnivorous Seed Plants

Thus far in this chapter we have considered the mineral nutrition of plants. As you already know, another crucial aspect of plant nutrition is photosynthesis—the principal

PLANT NUTRITION 643

36.9 The Nitrogen Cycle

Nitrogen fixation, nitrification, nitrate reduction, and denitrification are the components of an essential chemical cycle.

source of energy and carbon for plants themselves and for the biosphere as a whole. Not all plants, however, are photosynthetic autotrophs. A few, in the course of their evolution, have lost the ability to sustain themselves by photosynthesis. How do these plants get their energy and carbon?

A few plants are parasites that obtain their food directly from the living bodies of other plants. Perhaps the most familiar parasitic plants are the mistletoes and dodders (Figure 36.10). Mistletoes are green and carry on some photosynthesis, but they parasitize other plants for water and mineral nutrients and may derive photosynthetic products from them as well. Mistletoes and dodders extract nutrients from the vascular tissues of their hosts by forming absorptive organs called haustoria, which invade the host plant's tissue. Another parasitic plant, the Indian pipe, once was thought to obtain its food from dead organic matter. It is

Bacteria fix nitrogen from the atmosphere and conduct nitrification.



Tendrils of dodder

Dodder flowers

Host stem

36.10 A Parasitic Plant

Tendrils of dodder wrap around other plants. This parasitic plant obtains water, sugars, and other nutrients from its host through tiny, rootlike protuberances that penetrate the surface of the host.

644 CHAPTER THIRTY-SIX



36.11 A Carnivorous Sundew

Sundews trap insects on their sticky hairs. Secreted enzymes will digest the carcasses externally.

now known to get its nutrients, with the help of fungi, from nearby actively photosynthesizing plants. Hence it too is a parasite.

Some other plants that do not live by photosynthesis alone are the 450 or so carnivorous species—those that augment their nitrogen and phosphorus supply by capturing and digesting flies and other insects (Figure 36.11; also shown at the start of this chapter). The best-known carnivorous plants are Venus flytraps (genus *Dionaea*), sundews (genus *Drosera*), and pitcher plants (genus *Sarracenia*).

Carnivorous plants are normally found in boggy regions where the soil is acidic. Most decay-causing organisms require a less acidic pH to break down the bodies of dead organisms, so relatively little nitrogen is recycled into these acidic soils. Accordingly, the carnivorous plants have adaptations that allow them to augment their supply of nitrogen by capturing animals and digesting their proteins.

Sarracenia produces pitcher-shaped leaves that collect small amounts of rainwater. Insects are attracted into the pitchers either by bright colors or by scent and are prevented from getting out again by stiff, downward-pointing hairs. The insects eventually die and are digested by a combination of enzymes and bacteria in the water. Even rats have been found in large pitcher plants.

Sundews have leaves covered with hairs that secrete a

clear, sticky, sugary liquid. An insect touching one of these

hairs becomes stuck, and more hairs curve over the insect

and stick to it as well. The plant secretes enzymes to digest

insect and later absorbs the carbon- and nitrogen-

; products of digestion.

None of the carnivorous plants must feed on insects. They grow adequately without insects, but in their natural habitats they grow faster and are a darker green when they succeed in catching insects. The additional nitrogen from the insects is used to make more proteins, chlorophyll, and other nitrogen-containing compounds.

Chapter Summary

The Acquisition of Nutrients

- ▶ Plants are photosynthetic autotrophs that can produce all the compounds they need from carbon dioxide, water, and minerals, including a nitrogen source. They obtain energy from sunlight, carbon dioxide from the atmosphere, and nitrogen-containing ions and mineral nutrients from the soil.
- ▶ Plants explore their surroundings by growing rather than by locomotion.

Mineral Nutrients Essential to Plants

- ▶ Plants require 14 essential mineral elements, all of which come from the soil. Several essential elements fulfill multiple roles. Review Table 36.1
- ▶ The six mineral nutrients required in substantial amounts are called macronutrients; the eight required in much smaller amounts are called micronutrients. Review Table 36.1
- ▶ Deficiency symptoms suggest what essential element a plant lacks. Review Table 36.2
- ▶ Biologists discovered the essentiality of each mineral nutrient by growing plants on nutrient solutions lacking the test element. Review Figure 36.2

Soils and Plants

- ▶ Soils are complex in structure, with living and nonliving components. They contain water, gases, and inorganic and organic substances. They typically consist of two or three horizontal zones called horizons. Review Figures 36.3, 36.4, and Table 36.3
- ▶ Soils form by mechanical and chemical weathering of rock.
- ▶ Plants obtain some mineral nutrients by ion exchange between the soil solution and the surface of clay particles. Review Figure 36.5
- ▶ Farmers use fertilizer to make up for deficiencies in soil mineral nutrient content, and they apply lime to raise low soil pH.
- ▶ Plants affect soils in various ways, helping them form, adding material such as humus, and removing nutrients (especially in agriculture).

Nitrogen Fixation

- ▶ A few species of soil bacteria are responsible for almost all nitrogen fixation. Some nitrogen-fixing bacteria live free in the soil; others live symbiotically as bacteroids within the roots of plants.
- ▶ In nitrogen fixation, nitrogen gas (N_2) is reduced to ammonia (NH_3) or ammonium ions (NH_4^+) in a reaction catalyzed by nitrogenase. Review Figure 36.7
- ▶ Nitrogenase requires anaerobic conditions, but the bacteroids in root nodules require oxygen for their respiration. Leghemoglobin helps maintain the oxygen supply to the bacteroids.
- ▶ The formation of a nodule requires an interaction between the root system of a legume and Rhizobium bacteria. Review Figure 36.8

PLANT NUTRITION 645

- ▶ Nitrogen-fixing bacteria reduce atmospheric N_2 to ammonia, but most plants take up both ammonium ions and nitrate ions. Nitrifying bacteria oxidize ammonia to nitrate. Plants take up nitrate and reduce it back to ammonia, a feat of which animals are incapable. Review Figure 36.9
- ▶ Denitrifying bacteria return N_2 to the atmosphere, completing the biological nitrogen cycle. Review Figure 36.9

Sulfur Metabolism

- ▶ Plants take up sulfate ions and reduce them, forming the amino acids cysteine and methionine. Cysteine is the major precursor for other sulfur-containing compounds in plants and in animals, which must obtain their organic sulfur from plants.

Heterotrophic and Carnivorous Seed Plants

- ▶ A few heterotrophic plants are parasitic on other plants.

- Carnivorous plant species are autotrophs that supplement their nitrogen supply by feeding on insects.

For Discussion

1. Methods for determining whether a particular element is essential have been known for more than a century. Since these methods are so well established, why was the essentiality of some elements discovered only recently?
2. If a Venus flytrap were deprived of soil sulfates and hence made unable to synthesize the amino acids cysteine and methionine, would it die from lack of protein?
3. Soils are dynamic systems. What changes might result when land is subjected to heavy irrigation for agriculture after being relatively dry for many years? What changes in the soil might result when a virgin deciduous forest is cut down and replaced by crops that are harvested each year?
4. We mentioned that important positively charged ions are held in the soil by clay particles, but other, equally important, negatively charged ions are leached deeper into the soil's B horizon. Why doesn't leaching cause an electrical imbalance in the soil? (Hint: Think of the ionization of water.)
5. The biosphere of Earth as we know it depends on the existence of a few species of nitrogen-fixing prokaryotes. What do you think might happen if one of these species were to become extinct? If all of them were to disappear?

37

^J I Plant Growth Regulation



More than a century ago, Charles Darwin and his son studied the growth of plant shoots toward the light. Their findings, which we will detail in this chapter, pointed the way to the eventual discovery of the photoreceptor molecules that capture light signals and the hormones that transmit those signals to other parts of the plant. Light and hormones affect processes in plants as diverse as stem growth, flowering, bud dormancy, and the dropping of leaves in autumn. Several of the hormones now find important commercial applications, including the regulation of fruit ripening and enhanced germination of barley for the brewing industry.

Recent advances in understanding plant development have come largely from work with *Arabidopsis thaliana*, a little mustard-like weed. This plant is useful to researchers because its body and seeds are tiny, and its genome is unusually small for a flowering plant. It also flowers and forms seeds in a relatively short time after growth begins. *Arabidopsis* mutants with altered developmental patterns provide evidence for the existence of hormones and for the mechanisms of hormone and photoreceptor action.

In this chapter we first give a brief overview of the life of a flowering plant and its developmental stages. We explore the environmental cues, photoreceptors, and hormones that regulate plant development, and consider the multiple roles that each plays in normal development.

Interacting Factors in Plant Development

The development of a plant—the series of progressive changes that take place throughout its life—is regulated in complex ways. Four factors take part in this regulation:

- The plant senses and responds to environmental cues.
- The plant's genome encodes enzymes that catalyze the biochemical reactions of development, including the ones that make hormones and receptors, produce chemical building blocks, and participate in protein synthesis and energy metabolism.
- In order to sense environmental cues, the plant uses receptors, such as photoreceptors that absorb light.
- Chemical messages, or hormones, mediate the effects of the environmental cues sensed by the receptors.

Several hormones and photoreceptors regulate plant growth

Hormones are regulatory compounds that act at very low concentrations at sites distant from where they are produced. They mediate many developmental phenomena in plants, such as stem growth and autumn leaf fall. Unlike

Catching Some Rays

Most of us have observed the manner in which plants turn toward sunlight. Light signals caught by photoreceptor proteins are transmitted by hormones to other parts of the plant in a finely tuned developmental dance.



PLANT GROWTH REGULATION 647

J/,i Plant Hormones

HORMONE

TYPICAL ACTIVITIES

Abscisic acid Auxin

Brassinosteroids Cytokinins Ethylene Gibberellins

Jasmonates Oligosaccharins

Salicylic acid Systemin

Maintains seed dormancy and winter dormancy; closes stomata

Promotes stem elongation, adventitious root initiation, and fruit growth; inhibits lateral bud outgrowth and leaf abscission

Promote elongation of stems and pollen tubes; promote vascular tissue differentiation

Inhibit leaf senescence; promote cell division and lateral bud outgrowth; affect root growth

Promotes fruit ripening and leaf abscission; inhibits stem elongation and gravitropism

Promote seed germination, stem growth, and fruit development; break winter dormancy; mobilize nutrient reserves in grass seeds

Trigger defenses against pathogens and herbivores

Trigger defenses against pathogens; limit effects of high auxin concentrations; regulate cell differentiation

Triggers resistance to pathogens

Causes jasmonate production in response to tissue damage

animals, which produce each hormone in a specific part of the body, plants produce hormones in many of their cells. Each plant hormone plays multiple regulatory roles, affecting several different aspects of development (Table 37.1). Interactions among the hormones are often complex.

Like hormones, photoreceptors regulate many developmental processes in plants. Unlike the hormones, which are small molecules, plant photoreceptors are proteins. Light (an environmental cue) acts directly on photoreceptors, which in turn regulate processes such as the many changes accompanying the growth of a young plant out of the soil and into the light.

No matter what cues direct development, ultimately the plant's genome determines the limits within which the plant and its parts will develop. The genome encodes the master plan, but its interpretation depends on conditions in the environment. It is also the target for some hormone actions. For several decades hormones and photoreceptors were the focus of most work on plant development, but recent advances in molecular genetics allow us to focus on underlying processes such as signal transduction pathways.

Signal transduction pathways mediate hormone and photoreceptor action

We introduced the topic of signal transduction pathways in Chapter 15. Plants, like other organisms, make extensive use of these pathways. Cell signaling in plant development involves three steps: a receptor (for a hormone or for light), a signal transduction pathway, and the ultimate cellular response (see Figure 15.3). Protein kinase cascades amplify responses to receptor binding in plants, as they do in other organisms (see Figure 15.11). The signal transduction pathways of plants differ from those of animals only in the details; for example, their protein kinases

phosphorylate the amino acid residues serine or threonine but not tyrosine.

Before concerning ourselves with molecular details, let's set a broader context. What is the general pattern of plant

development?

From Seed to Death: An Overview of Plant Development

Let's review the life history of a flowering plant, from seed to death, focusing on how the developmental events are regulated. As plants develop, environmental cues, photoreceptors, and hormones affect three fundamental processes: cell division, cell expansion, and cell differentiation.

The seed germinates and forms a growing seedling

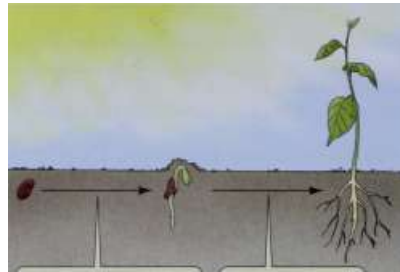
All developmental activity may be suspended in a seed, even when conditions appear to be suitable for its growth. In other words, a seed may be dormant. Typically, only 5 to 20 percent of a seed's weight is water, whereas most plant parts contain far more water.

Cells in dormant seeds do not divide, expand, or differentiate. For the embryo to begin developing, seed dormancy must be broken by one of several physical mechanisms, such as exposure to light, mechanical abrasion, fire, or leaching of inhibitors by water.

As the seed germinates (begins to develop), it first imbibes (takes up) water. The growing embryo must then obtain building blocks—monomers—for its development by digesting the polysaccharides, fats, and proteins stored in the cotyledons or in the endosperm. The embryos of some plant species secrete hormones that direct the mobilization of these reserves.

If the seed germinates underground, the new seedling must elongate rapidly and cope with life in darkness or dim

648 CHAPTER THIRTY-SEVEN



^

Dormancy may be overcome by abrasion, fire, light, leaching of inhibitors, or gibberellins.

Hormones and photoreceptors regulate seedling development.

37.1 From Seed to Seedling

Environmental factors, hormones, and photoreceptors regulate the first stages of plant growth.

light. A photoreceptor controls this stage, and ends it when the shoot is exposed to sufficient light to begin photosynthesis (Figure 37.1).

Early shoot development varies among the flowering plants. Figure 37.2 presents the distinctive shoot development patterns of monocots and eudicots. Plant growth from seedling to adult, both in darkness and in light, also involves several hormones.

The plant flowers and sets fruit

Flowering—the formation of reproductive organs—may be initiated when the plant reaches an appropriate age or size.

(a) Monocots

Q A coleoptile covers the early shoot of corn and other monocots, protecting it as the shoot grows to the soil surface.

(b) Eudicots (bean)

Some plant species, however, flower at particular times of the year, meaning that the plant must sense the appropriate time. In these plants, the leaves measure the length of the night (shorter in summer, longer in winter) with great precision. Light absorbed by photoreceptors affects this time-measuring process.

Once a leaf has determined that it is time for the plant to flower, that information must be transported as a signal to the places where flowers will form. The means by which this signal is transmitted remains a mystery, but it is likely that a "flowering hormone" travels from the leaf to the point of flower formation.

After flowers form, hormones play further roles. Hormones and other substances control the growth of a pollen tube down the style of a pistil. Following fertilization, a fruit develops and ripens under hormonal control (Figure 37.3).

The plant senesces and dies

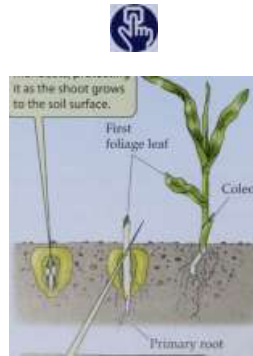
Some plants, known as perennials, continue to grow year after year. Many perennials have buds that enter a state of winter dormancy during the cold season. A hormone called abscisic acid helps maintain this dormancy.

In many species, leaves senesce (deteriorate because of aging) and fall at the end of the growing season, shortly before the onset of the severe conditions of winter. Leaf fall (abscission) is regulated by an interplay of the hormones ethylene and auxin. Finally, the entire plant senesces and dies.

fc 37.2 Patterns of Early Shoot Development

(a) In grasses and some other monocots, growing shoots are protected by a coleoptile until they reach the surface. (b) In most eudicots, the growing point of the shoot is protected by the cotyledons, (c) In some other eudicots, the cotyledons remain in the soil, and the growing point is protected by the first true leaves.

(c) Eudicots (pea)

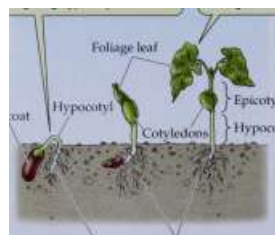


Q The shoot apex of most eudicots, such as the bean shown here, is protected by the cotyledons as the upper part of the plant is pulled through the soil by the elongating hypocotyl.

Q When the epicotyl elongates, the first foliage leaves emerge.

Coleoptile

Seed coat



Q In other eudicots, such as peas, the shoot apex is pulled up as the bent epicotyl elongates.

Epicotyl Hypocotyl

Q After the shoot emerges from the soil, it pierces the surrounding coleoptile and grows out.

Primary Secondary root roots



f

The cotyledons of peas remain in the soil.

f1An appropriate | night length may I trigger flowering.



I Photoreceptors and flowering hormone regulate flowering.



Auxin, gibberellins, and ethylene regulate fruit formation.



Vegetative plant

Flowering

Fruit formation

37.3 Flowering and Fruit Formation

Environmental cues, photoreceptors, and hormones regulate plant reproduction.

Death, which may be initiated by signals from the environment, follows senescent changes that are controlled by hormones such as ethylene. This life history pattern appears to be an adaptation for producing more offspring by pumping energy (food) and nutrients into the seeds; in so doing, the parent plant essentially starves itself to death.

We have reached the end of the plant's life history. Now let's examine how the various steps are regulated. We'll begin with regulation at the start of the life history—the seed and its germination.

Ending Seed Dormancy and Beginning Germination

The seeds of some species are, in effect, instant plants: All they need for germination is water. But many other species have seeds whose germination is regulated in more complex ways.

Seed dormancy may last for weeks, months, years, or even centuries. The mechanisms of dormancy are numerous and diverse, but three principal strategies dominate:

- ▶ Exclusion of water or oxygen from the embryo by means of an impermeable seed coat
- ▶ Mechanical restraint of the embryo by means of a tough seed coat
- ▶ Chemical inhibition of embryo development

The dormancy of seeds with impermeable coats can be broken if the seed coat is abraded as the seed tumbles across the ground or through creek beds, or passes through the digestive tract of an animal. Soil microorganisms probably play a major role in softening seed coats. Fire can release mechanical restraint. It can also melt wax in seed coats, removing the waterproofing and allowing water to reach the embryo (Figure 37.4). Leaching —prolonged exposure to water—is one way to reduce the level of a water-soluble chemical inhibitor and end dormancy. Scorching of seeds by fire can also break down some inhibitors.

PLANT GROWTH REGULATION 649

Seed dormancy affords adaptive advantages

What are the potential advantages of seed dormancy? For many species, dormancy assures survival through unfavorable

conditions and results in germination when conditions are more favorable. To avoid germination in the dry days of late summer, for example, some seeds must be exposed to a long cold period before they will germinate. Other seeds will not germinate until a certain amount of time has passed, regardless of how they are treated. This strategy prevents germination while the seed of a cereal grain, for example, is still attached to the parent plant.

Seeds that must be scorched by fire in order to germinate avoid competition by germinating only when an area has been cleared by fire. Light-requiring seeds, which germinate only at or near the surface of the soil, are generally tiny seeds with few food reserves. Conversely, germination of some seeds is inhibited by light; these seeds germinate only when buried and thus kept in darkness. Light-inhibited seeds are usually large and well stocked with nutrients.

Seed dormancy helps annual plants counter the effects of year-to-year variation in the environment. The seeds of some annuals remain dormant throughout an unfavorable year. The seeds of other plants germinate at different times



37.4 Fire and Seed Germination

This fireweed germinated and flourished after a great fire along the Alaska Highway.

650 CHAPTER THIRTY-SEVEN



37.5 Leaching of Germination Inhibitors

The seeds of the cypress, a swamp-adapted tree, germinate only after being leached by water, which increases the chances that they will germinate in a situation suitable for their growth.

during the year, increasing the likelihood that at least some of the seedlings will encounter favorable conditions.

Dormancy may also increase the likelihood of a seed's germinating in the right place. Some cypress trees, for example, grow in standing water, and their seeds germinate only if inhibitors are leached by water (Figure 37.5).

Seed germination begins with the uptake of water

The first step in seed germination is the uptake of water, called imbibition. A seed's water potential (see Chapter 35) is very negative, and water can be taken up readily if the seed coat allows it. The magnitude of this water potential is demonstrated by the force exerted by seeds expanding in water. Cocklebur seeds that are imbibing can exert a pressure of up to 1,000 atmospheres (about 100 megapascals) against a restraining force.

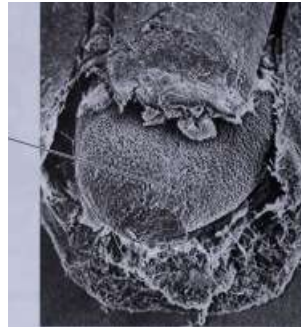
As a seed takes up water, it undergoes metabolic changes: Certain existing enzymes become activated, RNA and then proteins are synthesized, the rate of cellular respiration increases, and other metabolic pathways become activated. In many seeds there is no DNA synthesis and no cell division during these early stages of germination. Initially, growth results solely from the expansion of small, preformed cells. DNA is synthesized only after the embryonic root, called the radicle, begins to

grow and poke out beyond the seed coat (Figure 37.6).

The embryo must mobilize its reserves

Until the young plant (the seedling) becomes able to photo-synthesize, it depends on reserves stored in the endosperm or cotyledons. The principal reserve of energy and carbon

Radicle



37.6 The Radicle Emerges

The tip of this barley seed's radicle has just broken through its protective sheath. The appearance of the radicle—the embryonic root—is one of the first externally visible events in seed germination.

in many seeds is starch. Other seeds store fats or oils. Usually, the endosperm of the seed holds amino acid reserves in the form of proteins, rather than as free amino acids.

The giant molecules of starch, lipids, and proteins must be digested by enzymes into monomers that can enter the cells of the embryo. The polymer starch yields glucose for energy metabolism. The digestion of reserve proteins provides the amino acids the embryo needs to synthesize its own proteins. The digestion of lipids releases glycerol and fatty acids, both of which can be metabolized for energy. Glycerol and fatty acids can also be converted to glucose, which permits fat-storing plants to make all the building blocks they need for growth.

In germinating barley and other cereal seeds, the embryo secretes gibberellins, one of several classes of plant growth hormones. Gibberellins diffuse through the endosperm to a surrounding tissue called the aleurone layer, which lies inside the seed coat. The gibberellins trigger a crucial series of events in the aleurone layer, culminating in the release of enzymes that digest proteins and starch stored in the endosperm (Figure 37.7). Commercially, gibberellins are used in the brewing industry to enhance the "malting" (germination) of barley and the breakdown of its endosperm, producing sugar that is fermented to alcohol.

Gibberellins: Regulators from Germination to Fruit Growth

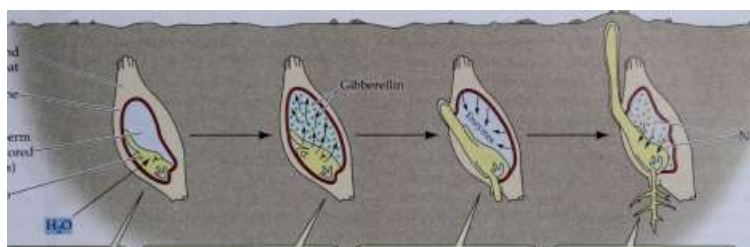
Gibberellins produce a wide variety of effects on plant development in addition to triggering digestive enzyme synthesis. We begin our discussion of the different plant growth hormones by discussing the discovery of the gibberellins, as well as their many effects.

Fruit and seed coat

Aleurone layer

Endosperm (with stored reserves)

Embryo



Nutrients

H₂O

The embryo imbibes H₂O and swells.

The embryo secretes gibberellins that diffuse into the aleurone layer and trigger the digestion of proteins to amino acids.

From the amino acids, digestive enzymes are synthesized; and the enzymes, along with other enzymes from the aleurone,

move into the endosperm.

The enzymes digest the reserve polymers in the endosperm, releasing monomers—nutrients from which the embryo synthesizes new cells.



37.7 Embryos Mobilize Polymer Reserves

Seed germination in cereal grasses consists of a cascade of processes. Gibberellins trigger the conversion of reserve polymers into monomers that can be used by the developing embryo.

Foolish seedlings led to the discovery of the gibberellins

The gibberellins are a large family of closely related compounds. Some are found in plants and others in a pathogenic (disease-causing) fungus, where they were first discovered.

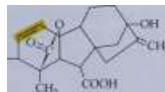
HO



HO

CH₃

COOH



COOH

Gibberellin A1

(important in stem growth)

Gibberellin A3 (commercially available)

In 1809, the study of the gibberellins began indirectly with observations of the bakanae, or "foolish seedling," disease of rice. Seedlings affected by this disease grow tall more rapidly than their healthy neighbors, but this rapid growth gives rise to spindly plants that die before producing seed (the rice grain used for food). The disease has had considerable economic impacts in several parts of the world. It is caused by the ascomycete fungus *Gibberella fujikuroi*.

In 1925, the Japanese biologist Eiichi Kurosawa grew *G. fujikuroi* on a liquid medium, then separated the fungus from the medium by filtering. He heated the filtered medium to kill any remaining fungus, but the resulting heat-treated filtrate still caused rapid growth in rice seedlings. Medium that had never contained the fungus did not stimulate seedling growth. This experiment established that *G. fujikuroi* produces a growth-promoting chemical substance, which Kurosawa called a gibberellin.

Were the gibberellins simply exotic products of an obscure fungus, or did they play a more general role in the

growth of plants? Bernard O. Phinney of the University of California, Los Angeles, answered this question in part in 1956, when he reported the spectacular growth-promoting effect of gibberellins on dwarf corn seedlings. He used plants that were known to be genetic dwarfs; each phenotype was produced when a particular recessive allele (say, *dl*) was present in the homozygous condition (*dl/dl*). Gibberellins applied to nondwarf—normal—corn seedlings had almost no effect, but gibberellins applied to the dwarfs caused them to grow as tall as their normal relatives. (A comparable effect of gibberellins applied to a dwarf tomato plant is shown in Figure 37.8.)

22 days after being sprayed with a tiny amount of gibberellin, this plant reached the size of a nondwarf plant



37.8 The Effect of Gibberellins on Dwarf Plants

In this experiment, the effect of gibberellins was tested on two dwarf tomato plants. Both plants were the same size when the one on the right was treated with gibberellins.

652 CHAPTER THIRTY-SEVEN

,-j'i

This result suggested to Phinney (1) that gibberellins are normal constituents of corn, and perhaps of all plants, and (2) that dwarf plants are short because they cannot produce their own gibberellins. According to Phinney's hypothesis, nondwarf plants manufacture enough gibberellins to promote their full growth, but dwarf plants do not. Extracts from numerous plant species were found to promote growth in dwarf corn. These findings provided direct evidence that plants that are not genetic dwarfs contain gib-berellin-like substances. Phinney's work set the stage for today's use of mutant plants to investigate the control of plant development.

The roots, leaves, and flowers of a dwarf corn plant appear normal, but the stems are much shorter than those of wild-type plants. All parts of the dwarf plant contain a much lower concentration of gibberellins than do the organs of a wild-type plant. We may infer, then, that stem elongation requires gibberellins or the products of gib-berellin action. We can further conclude that gibberellins play a less essential role in the development of roots, leaves, and flowers.

Although more than 80 gibberellins have been identified, only one, gibberellin A v actually controls stem elongation in most plants. The other gibberellins found in stems are simply intermediates in the production of gibberellin A v As we will see in the next section, gibberellins affect processes other than stem elongation, but we do not yet know which gibberellin has any other particular effect.

The gibberellins have many effects

Gibberellins and other hormones regulate the growth of fruits. It has long been known that seedless grapes (an inbred strain) form smaller fruit than their seeded relatives. Experimental removal of seeds from very young seeded grapes prevented normal fruit growth, suggesting that the seeds are sources of a fruit growth regulator. It was then shown that spraying young seedless grapes with a gibberellin solution caused them to grow as large as seeded ones. It is now a standard commercial procedure to spray seedless grapes with gibberellins. Subsequent biochemical studies showed that the developing seeds produce gibberellins, which diffuse out into the immature fruit tissue.

Some biennial plants respond dramatically to an increased level of gibberellins. Biennials grow vegetatively in their first year and flower and die in their second year. In the second year, the apical meristems of biennials respond to environmental cues by producing elongated shoots that eventually bear flowers. This elongation is called bolting. When the plant senses the appropriate environmental cue—longer days or a sufficient winter chilling—it produces more gibberellins, raising the gibberellin concentration to a level that causes the shoot to bolt. Plants of some biennial species will bolt when sprayed with a gibberellin solution without the environmental cue (Figure 37.9).

Gibberellins also cause fruit to grow from unfertilized flowers, promote seed germination in lettuce and some other species, and help bring spring buds out of winter dor-

37.9 Bolting

Spraying with gibberellins causes cabbage and some other plants to bolt.

The internodes of plants treated with gibberellin elongate dramatically, resulting in towering shoots.

Untreated control plants retain their compact, leafy heads.



Without gibberellin

With gibberellin

mancy. Most hormones have multiple effects within the plant, and they often interact with one another in regulating developmental processes. In controlling stem elongation, for example, gibberellins interact with another hormone, auxin.

Auxin Affects Plant Growth and Form

If you pinch off the apical bud at the top of a bean plant, inactive lateral buds become active, developing into branches. Similarly, pruning a shrub causes an increase in branching. If you cut off the blade of a leaf but leave its petiole (stalk) attached to the plant, the petiole drops off sooner than it would have if the leaf were intact. If a plant is kept indoors, its shoot system grows toward a window. These diverse responses of shoot systems are all mediated by a plant hormone called auxin, or indoleacetic acid (IAA).



$\text{H}_2\text{C}-\text{COOH}$

Auxin (indoleacetic acid)

In the discussions that follow, we will look at the discovery of auxin, its transport within the plant, and its role as mediator of the effects of light and gravity on plant growth. We'll discover its many effects on vegetative growth and on fruit development. Then we'll examine its mechanism of action.

Plant movements led to the discovery of auxin

The discovery of auxin and its numerous physiological effects can be traced back to work done in the 1880s by Charles Darwin and his son Francis. The Darwins were interested in plant movements. One type of movement they studied was phototropism, the growth of plant structures toward light (as in most shoots) or away from it (as in roots). They asked, What part of the plant senses the light? To answer this question, the Darwins worked with canary grass (*Phalaris canariensis*) seedlings grown in the dark. A young grass seedling has a coleoptile —a cylindrical

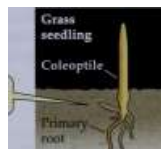
EXPERIMENT

Question: Where is the light receptor for phototropism?

METHOD

f

Grass seedlings were grown in the dark.



f

The seedlings were "blindfolded" and exposed to light on one side.

Coleoptile

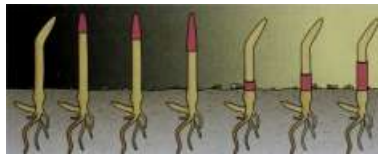
Blindfold

Light



V v }f V V V }f

RESULTS



o Coleoptiles responded to light only when the tip was exposed.

Conclusion: The plant's "eye" (the part that senses light) is in the tip, and it sends a message from the tip to the region of bending.

sheath a few cells thick that protects the delicate shoot as it pushes through the soil (see Figure 37.2a). When the coleoptile breaks through the surface of the soil, it soon stops growing, and the first leaves emerge unharmed. The coleoptiles of grasses are phototropic— they grow toward the light.

To find the light-receptive region of the coleoptile, the Darwins tried "blindfolding" the coleoptiles of dark-grown canary grass seedlings in various places, then illuminating them from one side (Figure 37.10). The coleoptile grew toward the light whenever its tip was exposed. If the top millimeter or more of the coleoptile was covered, however, there was no phototropic response. Thus the tip contains the photoreceptor that responds to light. The actual bending toward the light, however, takes place in a growing region a few millimeters below the tip. Therefore, the Darwins reasoned, some type of message must travel within the coleoptile from the tip to the growing region. Others later demonstrated that the message is a chemical substance by showing that it can move through certain nonliving materials, such as gelatin, but not through others, such as a metal barrier.

Further experiments showed that the tip of the coleoptile produces a hormone that moves down the coleoptile to the growing region. If the tip is removed, the growth of the coleoptile is sharply inhibited. If the tip is carefully replaced, growth resumes, even if the tip and base are separated by a thin layer of gelatin. The hormone moves down from the tip, but it does not move from one side of the coleoptile to the other. If the tip is cut off and replaced so that it covers only one side of the cut end of the coleoptile, the coleoptile curves as the cells on the side below the replaced tip grow more rapidly than those on the other side.

The Dutch botanist Frits W. Went removed coleoptile tips and placed their cut surfaces on a block of gelatin. Then he placed pieces of the gelatin block on decapitated coleoptiles—positioned to cover only one side, just as coleoptile tips had been placed in earlier experiments (Figure 37.11). As they grew, the coleoptiles curved toward the side away from the gelatin. This curvature demonstrated that a hormone had indeed diffused into the gelatin block from the isolated coleoptile tips. Went had at last isolated a hormone from a plant. Later chemical analysis showed that this hormone, named auxin, was indoleacetic acid.

Auxin transport is polar

Since being isolated, auxin has been intensively studied. Early experiments showed that its movement through certain plant tissues is strictly polar—that is, unidirectional along a line from apex to base. By inverting some plants or plant parts, scientists determined that the apex-to-base di-

\Ttim. 37.70 The Darwins'Phototropism Experiment

The top drawings show some of the ways in which seedlings grown in the dark were "blindfolded"; the lower drawings show what the Darwins observed in each case. Their observations led them to hypothesize the existence of a growth-promoting "messenger" substance produced by the coleoptile.



654 CHAPTER THIRTY-SEVEN

EXPERIMENT

Question: Can a growth hormone be isolated from the coleoptile tip?

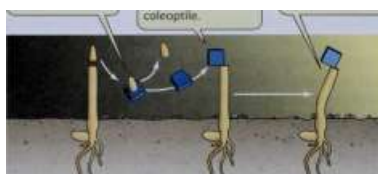
METHOD

RESULT

The tip is removed and placed on gelatin.

The gelatin is placed on one edge of another decapitated coleoptile.

The coleoptile curves away from the gelatin as it grows.



Oat coleoptile

Conclusion: A growth hormone diffused from the tip into the gelatin, and from the gelatin into another plant. It had an effect on the growth of the plants similar to that of a coleoptile tip.

37.77 Went's Experiment

Went succeeded in isolating the growth-promoting chemical substance whose existence the Darwins had hypothesized by placing coleoptile tips on a block of gelatin.

direction of auxin movement has nothing to do with gravity; the polarity of this movement is a totally biological matter. Many plant parts show complete or partial polarity of auxin transport. For example, in most leaf petioles auxin moves only from the blade end toward the stem end.

Auxin carrier proteins move auxin into and out of cells

In one of the most intense areas of current research on auxin, biologists are using *Arabidopsis* plants that have mutations affecting the transport of auxin. By cloning genes from these plants and characterizing their products, they are finding a growing number of auxin carrier proteins. In polar transport in the stem, a carrier protein imports auxin at the end of the cell toward the shoot apex, and it or another carrier exports auxin at the other end of the cell. Auxin carrier proteins contribute to the establishment of auxin gradients in the plant. As a result, auxin acts as a morphogen, telling cells where they lie within the plant and determining how they differentiate. There are probably auxin carrier proteins specific to different tissues and different cells, participating in different auxin responses.

Light and gravity affect the direction of plant growth

While polar auxin transport establishes the orientation of

auxin, (side-to-side) redistribution of auxin appears

! mechanism that explains both phototropism and

another response depending on differential growth, gravitropism. This redistribution may be carried out by other auxin carrier proteins.

When light strikes a coleoptile from one side, auxin at the tip moves laterally toward the shaded side. The imbalance thus established is maintained down the coleoptile, so that in the growing region below, there is more auxin on the shaded side, causing the unequal growth that results in a coleoptile bent toward the light. This bending toward light is phototropism (Figure 37.12a). If you have noticed a house plant bending and pointing toward a window, you have seen phototropism.

Even in the dark, auxin moves to the lower side of a shoot that has been tipped over, causing more rapid growth in the lower side and, hence, an upward bending of the shoot. Such growth in a direction determined by gravity is called gravitropism (Figure 37.12b). The upward gravitropic response of shoots is defined as negative; the gravitropism of roots, which bend downward, is positive.

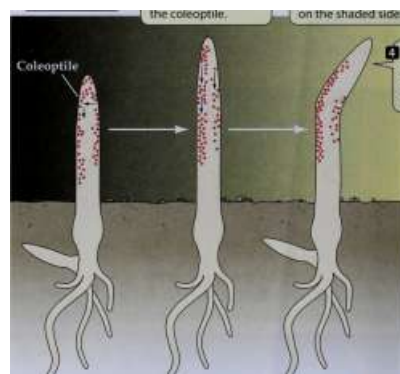
(a) Phototropism

f

Auxin moves to the shaded side.

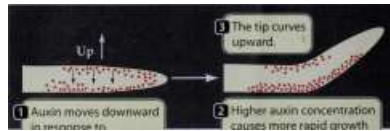
EJ Redistribution stops as auxin moves down the coleoptile.

Q Higher auxin concentration causes more rapid growth on the shaded side.



The tip curves toward the light.

(b) Gravitropism



I Auxin moves downward in response to gravitational stimulus.

I Higher auxin concentration causes more rapid growth on the lower side.

rn



37.12 Plants Respond to Light and Gravity

Phototropism and gravitropism occur in response to a redistribution of auxin.

Auxin affects vegetative growth in several ways

Like the gibberellins, auxin has many roles in plant development. It affects the vegetative growth of plants in several ways, including:

- ▶ Initiating root growth in cuttings
- ▶ Stimulating the detachment of old leaves from their stems (abscission)
- ▶ Maintaining apical dominance
- ▶ Promoting stem elongation and inhibiting root elongation

Let's examine each of these aspects in turn.

Cuttings from the shoots of some plants can produce roots and grow into entire new plants. For this to happen, certain undifferentiated cells in the interior of the shoot, originally destined to function only in food storage, must set off on a new mission: They must differentiate and become organized into the apical meristem of a new root.

These changes are similar to those in the pericycle of a root when a lateral root forms (see Chapter 34). Shoot cuttings of many species can be stimulated to grow profuse roots by dipping the cut surfaces into an auxin solution; this observation suggests that the plant's own auxin plays a role in the initiation of lateral roots. Commercial preparations that enhance the rooting of plant cuttings typically contain mostly synthetic auxins.

The effect of auxin on the detachment of old leaves from stems is quite different from root initiation. This process, called abscission, is the cause of autumn leaf fall. Leaves consist of a blade and a petiole that attaches the blade to the stem. Abscission results from the breakdown of a specific part of the petiole, the abscission zone (Figure 37.13). If the blade of a leaf is cut off, the petiole falls from the plant more rapidly than if the leaf had remained intact. If the cut surface is treated with an auxin solution, however, the petiole remains attached to the plant, often longer than an intact leaf would have (Figure 37.14). The time of abscission of leaves in nature appears to be determined in part by a decrease in the movement of auxin, produced in the blade, through the petiole.

37.7 3 When a Leaf Is About to Fall

The breakdown of the abscission zone of the petiole causes the leaf to fall.



∴**

m^o^-

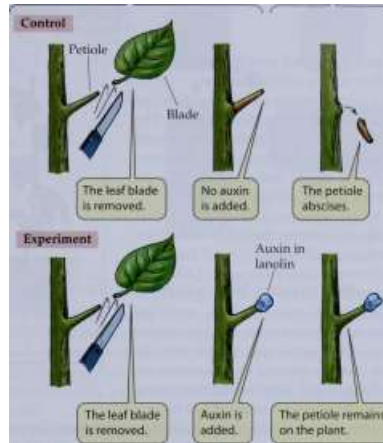
Abscission zone

EXPERIMENT

Question: How does auxin affect leaf abscission?

METHOD

RESULTS



The leaf blade is removed.

The petiole remains on the plant.

Conclusion: Auxin inhibits leaf abscission.

37.14 Auxin and Leaf Abscission

The leaf blade is a source of auxin throughout the growing season; without auxin, the petiole falls from the plant.

Auxin maintains apical dominance, a phenomenon in which apical buds inhibit the growth of lateral buds. This phenomenon can be demonstrated by an experiment with young seedlings. If the plant remains intact, the stem elongates, and the lateral buds remain inactive. Removal of the apical bud—the major site of auxin production—permits the lateral buds to grow out vigorously. If the cut surface of the stem is treated with an auxin solution, however, the lateral buds do not grow (Figure 37.15). Apical buds of branches also exert apical dominance: The lateral buds on the branch are inactive unless the apex of the branch is removed.

In the two experiments on leaves and stems that we have just discussed, removal of a particular part of the plant produces an effect—abscission or loss of apical dominance—and that effect is prevented by treatment with auxin. These results are consistent with other data showing that the excised part of the leaf or stem is an auxin source and that auxin in the intact plant helps maintain apical dominance and delays the abscission of leaves. As we will discover later, other hormones can modify the effects of auxin. Plant

656 CHAPTER THIRTY-SEVEN

37.15 Auxin and Apical Dominance

Auxin produced by the apical bud maintains apical dominance—the growth of a single main stem with minimal branching.

EXPERIMENT

Question: What is the role of auxin in apical dominance?

In intact plants, lateral buds are inhibited by the apical bud.

After the plant is decapitated, an agar block containing no auxin is applied to the stump.

Some lateral buds grow out.

growth is regulated more by hormone interactions than by a single hormone.

Auxin promotes stem elongation, but it inhibits the elongation of roots. The question of why different organs should respond in opposite ways to the same chemical signal remains unanswered, but is a subject of current research.

Many synthetic auxins—chemical analogs of indoleacetic acid—have been produced and studied. One of them, 2,4-dichlorophenoxyacetic acid (2,4-D), has the striking property of being lethal to eudicots at concentrations that are harmless to monocots. This property made 2,4-D an effective selective herbicide that could be sprayed on a lawn or a cereal crop to kill those weeds that are eudicots. However, because 2,4-D takes a long time to break down, it pollutes the environment, so scientists are seeking new approaches to selective weed killing.

Auxin controls the development of some fruits

Although fruit development normally depends on prior fertilization of the egg, in many species treatment of an unfertilized ovary with auxin or gibberellins causes parthenocarpy—fruit formation without fertilization of the egg. Parthenocarpic fruits form spontaneously in some plants, including dandelions, seedless grapes, and cultivated bananas.

All of these activities illustrate the great diversity of important roles that auxin plays. Now let's see how auxin plays one of its roles—promoting stem elongation through effects on the cell wall.



An agar block containing auxin is applied at the time of decapitation.

The lateral buds are inhibited.

New branch

Agar block New branch



Inhibited buds

Experimental plant: No auxin Experimental plant: Auxin

Conclusion: Apical dominance results from auxin produced by the apical bud.

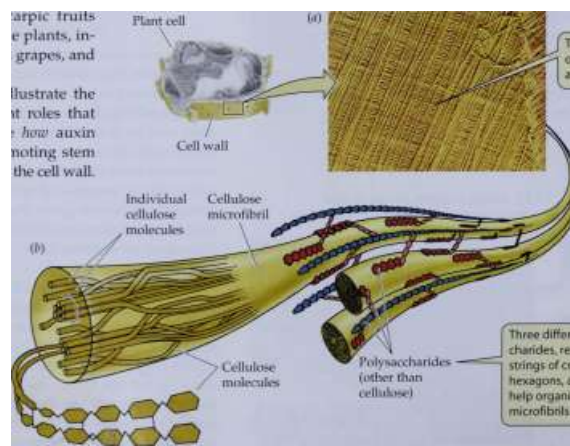
Auxin promotes growth by acting on cell walls

CELL WALLS ARE A KEY TO PLANT GROWTH. The principal strengthening component of the plant cell wall is cellulose, a large polymer of glucose. In the wall, cellulose molecules tend to associate in parallel with one another. Bundles of approximately 250 cellulose molecules make up microfibrils

Plant cell

37.76 Cellulose in the Cell Wall

- Plant cell wall is a net-of cellulose microfibrils linked by other polysaccharides.



The parallel microfibrils of cellulose associate in a crisscross pattern.

Polysaccharides (other than cellulose)

Three different polysaccharides, represented by strings of cones, hexagons, and ovals, help organize cellulose microfibrils in the wall.

/

that are visible with an electron microscope (Figure 37.16). What makes the cell wall rigid is a network of cellulose microfibrils connected by bridges of other, smaller polysaccharides (Figure 37.16b). The orientation of the cellulose microfibrils determines the direction of cell expansion (Figure 37.17).

The growth of a plant cell is driven primarily by the uptake of water, which enters the cytoplasm of the cell and accumulates in its central vacuole. As the vacuole expands, the cell grows rapidly, with the vacuole often making up more than 90 percent of the volume of a mature cell. As the vacuole expands, it presses the cytoplasm against the cell wall, and the wall resists this force.

For the cell to grow, its wall must loosen and be stretched. If the wall simply stretched, it would become thinner. However, new polysaccharides are deposited throughout the wall and new cellulose microfibrils are deposited at the inner surface of the wall, maintaining its thickness. Thus the cellulose microfibrils in the outermost part of the wall are the oldest, and those in the innermost part the youngest.

The cell wall plays key roles in controlling the rate and direction of growth of a plant cell. How does the plant determine the behavior of its cell walls?

Auxin loosens the cell wall. Experiments with segments of oat coleoptiles showed that plant cell walls recover incompletely from being stretched (Figure 37.18). Reversible stretching is called elasticity, and irreversible stretching is called plasticity. Pretreating the coleoptile segments with auxin significantly increased their plasticity; in other words, it loosened the cell walls. This result suggested that auxin-induced cell expansion might result from just such a loosening effect.

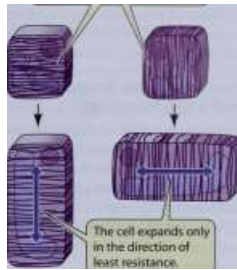
Auxin acts by causing the release of a "wall-loosening factor" from the cytoplasm. Studies in the 1970s indicated that the wall-loosening factor was sometimes simply hydrogen ions (protons, H^+). Acidifying the growth medium (that is, adding H^+) causes segments of stems or coleoptiles to grow as rapidly as segments treated with auxin. Furthermore, treating coleoptile segments with auxin causes acidification of the growth medium. Treatments that block acidification by auxin also block auxin-induced growth. It was suggested that hydrogen ions secreted into the cell wall as a result of auxin action might activate one or more proteins in the wall.

Proteins called expansins, isolated from plant cell walls in the 1990s, were found to cause the extension of isolated cell walls of several species. Expansins are widespread among land plants. Expansin action is pH-dependent, and the expansins appear to be activated by hydrogen ions. These proteins apparently modify hydrogen bonding between polysaccharides in the plant cell wall. The changed hydrogen bonding pattern may allow the polysaccharide macromolecules to slip past each other, so that the wall stretches and the cell expands.

37.77 Plant Cells Expand

The orientation of cellulose microfibrils in the plant's cell walls determines the direction of cell expansion.

Cellulose microfibrils encircle the cell in a specific orientation and constrain cell expansion.



The cell expands only in the direction of least resistance.

Plants contain specific auxin receptor proteins

The initial step in the action of any plant hormone is the binding of the hormone to specific receptor proteins. Several proteins can bind various plant hormones, but some of this binding may be nonspecific. It must be shown that auxin-binding proteins actually mediate the effects of auxin.

Plant molecular biologists showed that the protein ABP1 (Auxin-Binding Protein 1) functions as an auxin receptor. They inserted the ABP1 gene of Arabidopsis into other species and then induced the expression of the gene in cells that normally show a limited response to auxin. Upon expression of the inserted ABP1 gene, the cells showed

EXPERIMENT

Question: How does auxin affect cell walls?

METHOD

RESULTS

Control

Segment of

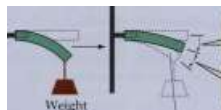
Pin

coleoptile

Weight added Weight removed

Experiment

Auxin-treated segment of coleoptile



Weight

Irreversible bending

is called plasticity.

. /

i .

Reversible bending

is called elasticity.



Position of coleoptile after weight removed

Plasticity Elasticity

Weight

Conclusion: Auxin loosens cell walls by increasing plasticity.

37.18 Auxin Affects Cell Walls

Auxin increases the plasticity, but not the elasticity, of cell walls.

658 CHAPTER THIRTY-SEVEN

greater responses to both endogenous and applied auxin. Subsequent work has conclusively shown the existence and importance of other auxin receptor proteins. Given the number of processes regulated by auxin, it is hardly surprising that there appear to be multiple receptors and signal transduction pathways for this hormone.

Auxin and other hormones evoke differentiation and organ formation

What plant substance signals the different types of cells and organs to form? Much of the research on such questions has been done with plant tissues grown in culture outside the plant body. One easily grown tissue is pith—the spongy, innermost tissue of a stem. Pith tissue cultures proliferate rapidly, but show no differentiation. All the cells are similar and unspecialized; they grow into a lump on the surface of a culture medium.

Cutting a notch in the cultured pith tissue and inserting a stem tip into the notch causes the pith cells below the inserted tip to differentiate. Some of them differentiate to form water-conducting xylem cells. Differentiation of pith cells can also be initiated by adding to the notch a mixture of auxin and coconut milk (a rich source of plant hormones).

A similar effect can be observed in intact plants. If notches are cut in the stems of *Coleus blumei* plants, interrupting some of the strands of vascular tissue, the strands gradually regenerate from the upper side of the cut to the lower (recall that auxin moves from the tip to the base of a stem). If the leaves above the cut are removed, regeneration is slowed. However, when the missing leaves are replaced with an auxin solution, vascular tissue regenerates. Auxin and other plant hormones signal the formation of specific cell types.

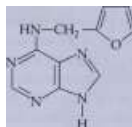
Experiments with cultured tissues have helped clarify which hormones control organ formation. Undifferentiated cultures of tobacco pith form roots when treated with an appropriate concentration of auxin. Another group of hormones—the cytokinins—causes buds and then shoots to form in such cultures. The pattern of organ formation depends on the ratio of auxin to cytokinin in the medium. A high proportion of auxin favors roots, and a high proportion of cytokinins favors buds, but both processes are most active when both hormones are present.

Cytokinins Are Active from Seed to Senescence

Besides stimulating bud formation, the cytokinins promote cell division in cultured plant tissues, an activity that led to their discovery. In addition, cytokinins aid germination, inhibit stem elongation, stimulate lateral bud growth, and delay leaf senescence.

Cytokinins are derivatives of adenine. In studies of plant cell division, botanists discovered a substance that powerfully stimulated cell division in tissue cultures. This compound, kinetin, consists of adenine with an attached group. We now know that kinetin is just one of a family of com-

pounds, which are now called cytokinins. Kinetin may be considered a synthetic cytokinin, because it has never been isolated from plant tissue. However, two closely related compounds, called zeatin and isopentenyl adenine, occur naturally in plants.



$\text{HN}-\text{CH}_2-\text{CH}=\text{c};$



.CH,

*CH₂OH

Kinetin

(a cytokinin discovered in aged DNA)

Zeatin

(a naturally occurring

cytokinin in plants)

Cytokinins form primarily in the roots and move to other parts of the plant. They have several effects:

► Adding an appropriate combination of auxin and cytokinins to a growth medium yields rapid growth of plant tissues.

- Cytokinins can cause certain light-requiring seeds to germinate when the seeds are kept in constant darkness.
- Cytokinins usually inhibit the elongation of stems, but they cause lateral swelling of stems and roots (the fleshy roots of radishes are an extreme example).
- Cytokinins stimulate lateral buds to grow into branches; thus the balance between auxin and cytokinin levels controls the bushiness of a plant.
- Cytokinins increase the expansion of cut pieces of leaf tissue, and may regulate normal leaf expansion.
- Cytokinins delay the senescence of leaves. If leaf blades are detached from a plant and floated on water or a nutrient solution, they quickly turn yellow and show other signs of senescence. If instead they are floated on a solution containing a cytokinin, they remain green and senesce much more slowly.

Ethylene: A Gaseous Hormone That Promotes Senescence

Whereas the cytokinins oppose or delay senescence, another plant hormone promotes it. This hormone is the gas ethylene, which is sometimes called the senescence hormone. Ethylene can be produced by all parts of the plant, and like all plant hormones, it has several effects.

Ethylene

(the "senescence hormone")

H

H

C=C

H

H

Back when streets were lit by gas rather than by electricity, leaves on trees near street lamps abscised earlier than those on trees farther from the lamps. We now know that ethylene, a combustion product of the illuminating gas, is what caused the abscission. Auxin delays leaf abscission, but ethylene strongly promotes it; thus a balance of auxin and ethylene controls abscission.

PLANT GROWTH REGULATION 659

Ethylene hastens the ripening of fruit

By promoting senescence, ethylene speeds the ripening of fruit. The old saying "one rotten apple spoils the barrel" is true. That rotten apple is a rich source of ethylene, which speeds the ripening and subsequent rotting of the others in the barrel. As the fruit ripens, it loses chlorophyll and its cell walls break down. Ethylene produced in the fruit tissue promotes both processes. Ethylene also causes an increase in its own production. Thus, once ripening begins, more and more ethylene forms, and because it is a gas, it diffuses readily throughout the fruit and even to neighboring fruits on the same or other plants.

Farmers in ancient times used to slash developing figs to hasten their ripening. We now know that wounding causes an increase in ethylene production by the fruit, and that the raised ethylene level promotes ripening. Today commercial shippers and storers of fruit hasten ripening by adding ethylene to storage chambers. This use of ethylene is the single most important use of a plant hormone in agriculture and commerce. Ripening can also be delayed by the use of "scrubbers" and adsorbents to remove ethylene from the atmosphere in fruit storage chambers.

As flowers senesce, their petals may abscise, to the detriment of the cut-flower industry. Florists or their suppliers often spray their flowers with dilute solutions of silver thio-sulfate. Silver salts inhibit ethylene action, probably by interacting directly with the ethylene receptor, and thus delay senescence—enabling florists to keep their wares salable longer.

Ethylene affects stems in several ways

Although associated primarily with senescence, ethylene is active at other stages of plant development as well. The stems of many eudicot seedlings form an apical hook that protects the delicate shoot apex while the stem grows through the soil (Figure 37.19). The apical hook is maintained through an asymmetrical production of ethylene gas, which inhibits the elongation of cells on the inner surface of the hook. Once the seedling breaks through the soil

surface and is exposed to light, ethylene synthesis stops, and the cells of the inner surface are no longer inhibited. These cells now elongate, and the hook opens, raising the shoot apex and expanding leaves into the sun.

37.79 The Apical Hook of a Eudicot

Asymmetrical production of ethylene is responsible for the apical hook of this seedling, which was grown in the dark.



Ethylene also inhibits stem elongation in general, promotes lateral swelling of stems (as do the cytokinins), and causes stems to lose their sensitivity to gravitropic stimulation.

The ethylene signal transduction pathway is well understood

Analysis of Arabidopsis mutants has revealed the steps in the mechanism of ethylene action. Some of these mutants do not respond to applied ethylene, and others act as if they have been exposed to ethylene even though they haven't. Studies of genes from these mutants and their protein products, coupled with comparisons of their amino acid sequences with those of other known proteins, have revealed some of the details of the signal transduction pathway through which ethylene produces its effects (Figure 37.20).

(a) Ethylene absent

(b) Ethylene present

|WhenETR1 is not active...



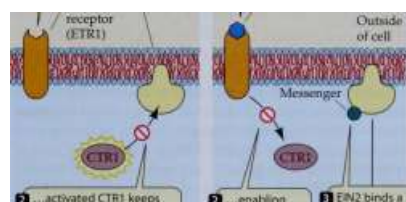
Ethylene binds its receptor, ETR1,...

^Ethylene EEST2 receptor / ^J\ (ETR1)



Ethylene

Outside of cell



...activated CTR1 keeps the membrane protein EIN2 inactive.

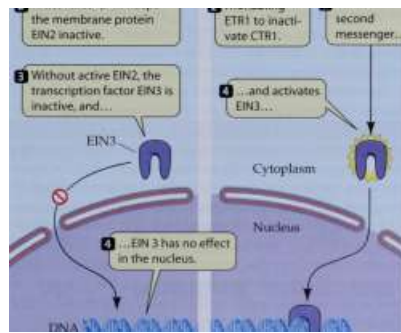
...enabling ETR1 to inactivate CTRL

EIN2bindsa

second

messenger...

Without active EIN2, the transcription factor EIN3 is inactive, and...



dna V\!%%:!!%^ . »/||Wir«



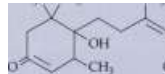
A

Q • .which turns on the expression of genes whose products lead to the physiological effects of ethylene.

37.20 A Signal Transduction Pathway for Ethylene

This slightly simplified scheme shows the roles of four gene products (ETR1, CTR1, EIN2, and EIN3) in the signal transduction pathway through which ethylene exerts its effects.

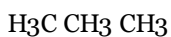
66o CHAPTER THIRTY-SEVEN



The pathway includes two membrane proteins: The first is an ethylene receptor (ETR1), and the second is a channel (EIN2) that acts through a second messenger to activate a transcription factor that turns on genes. The resolution of this pathway has been one of the high points of plant biological research over the last 30 years.

Absciscic Acid: The Stress Hormone

Absciscic acid is another hormone that has multiple effects in the living plant. During embryo formation, absciscic acid promotes the accumulation of storage proteins in seeds by allowing the expression of the genes that encode those proteins. It is generally present in high concentrations in dormant buds and some dormant seeds, and it is the most common inhibitor of seed germination. Absciscic acid also inhibits stem elongation. It is sometimes referred to as the stress hormone of plants because it accumulates when plants are deprived of water and because of its possible role in maintaining the winter dormancy of buds.



Absciscic acid

(the "stress hormone")

COOH

Some mutant corn plants, called vp mutants, have seeds that germinate while still attached to the cob, on the intact plant—a condition called vivipary. Several vp mutants are naturally deficient in absciscic acid, and a different kind of vp mutant fails to respond in any way to applied absciscic acid. Applying absciscic acid to the absciscic acid-deficient mutants reduces their tendency to show vivipary. The results of applying absciscic acid to both kinds of mutants indicate that it is the inhibitor that normally prevents seeds from germinating on the plant rather than in the soil.

Absciscic acid also regulates gas and water vapor exchange between leaves and the atmosphere through its effects on the guard cells of the leaf stomata (see Chapter 35). Absciscic acid causes stomata to close, and it also prevents stomatal opening normally caused by light. Both of these processes involve ion channels in the plasma membrane of the guard cells. The first response of a guard cell to absciscic acid is the opening of calcium channels and the entry of calcium into the cell. This calcium causes the cell's vacuole to release calcium, too. The increased concentration of calcium leads to a chain of events that result in the opening of potassium channels and the release of K⁺ and of water, and the closing of the stoma as the guard cells sag together.

Hormones in Plant Defenses

When bacteria, viruses, or fungi attack a plant, the plant responds in several ways, as we will see in Chapter 39. One of its first responses is to release hormones called oligosaccharins. These hormones, as their name implies, are oligosaccharides—compounds consisting of a few sugar or derivative sugar units. They are actually fragments of the cell wall,

which are released when enzymes from an attacker degrade it. They act as signals that trigger the plant's defenses.

Because auxin modifies the cell wall, it is not surprising that auxin, too, causes the release of oligosaccharins. The interactions between auxin and oligosaccharins may be complex. One oligosaccharin, at an extremely low concentration, has been found to inhibit auxin-induced growth promotion. Other oligosaccharins may regulate aspects of cell differentiation.

Three other hormones— jasmonates, salicylic acid, and sys-temin —serve as important signals in plant defenses. Their activities will be discussed in Chapter 39.

Brassinosteroids: "New" Hormones with Multiple Effects

More than 20 years ago, biologists isolated an interesting steroid from the pollen of rape, a member of the Brassi-caceae, or mustard family. When applied to various plant tissues, this brassinosteroid stimulated cell elongation, pollen tube elongation, and vascular tissue differentiation, and it inhibited root elongation. Since then, dozens of chemically related and growth-affecting brassinosteroids have been found in plants. Treatment with as little as a few nanograms of brassinosteroid per plant is enough to promote growth. However, the brassinosteroids were not at first regarded as plant hormones, in part because of similarities between their effects and those of auxin.

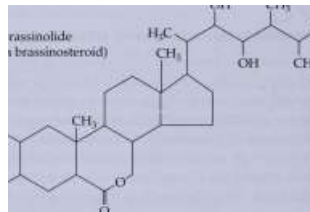
OH CH₃,

Brassinolide

(a brassinosteroid)

HO

HO



CH₃,

The properties of an Arabidopsis mutant called det1 made it clear that brassinosteroids are naturally occurring plant hormones. When grown in darkness, seedlings homozygous for the det1 allele differ dramatically from wild-type seedlings: In many respects, they look like wild-type seedlings grown in the light. Treatment of dark-grown det1 mutant seedlings with brassinosteroids causes them to grow normally—that is, like wild-type plants grown in the dark. The det1 plants are unable to synthesize their own brassinosteroids, and the lack of the hormone results in abnormal growth.

Brassinosteroids will probably be important in agriculture. They have increased the yields of some crops in field tests. Could they also be useful for keeping some plants small? What about limiting the growth rate of lawns and the height of trees and hedges? Joanne Chory and her colleagues at the University of California, San Diego and the

Salk Institute found a way to do this. They showed that a mutation of a gene called bas-1 in Arabidopsis results in a dwarfed plant because the gene's product inactivates brassinosteroids in the stem. By introducing the bas-1 mutation into selected plants, agriculturists could produce slow-growing plants and then adjust their growth rate by treatment with brassinosteroids.

Chory and others have shown that some of the effects of light on plant development result from effects on the signal transduction pathway for brassinosteroids. Let's now look more closely at the effects of environmental cues such as light.

Light and Photoreceptors

The length of the night determines the onset of winter dormancy. As summer wears on, the days become shorter (that is, the nights become longer). Leaves have a mechanism for measuring the length of the night, as we will see in the next chapter. Measuring night length is an accurate way to determine the season of the year. If a plant determined the season by the temperature, it could be fooled by a winter warm spell or by unseasonably cold weather in the summer. The length of the night, on the other hand, is determined by Earth's rotation around the sun and does not vary. Plants use the environmental cue of night length to time several aspects of their growth and development.

Length of the night is one of several environmental cues detected by plants, or by individual parts such as leaves. Light—its presence or absence, its intensity, its color, and its duration—provides cues to various conditions. Temperature, too, provides important environmental cues, both by its value at any particular time and by the distribution of warmer and colder stretches over a period of time. The plant "reads" an environmental cue and then "interprets" it, often by stepping up or decreasing its production of hormones.

We'll discuss an example of a temperature cue in the next chapter. Here, we'll see how certain photoreceptors interpret light, its duration, and its wavelength distribution.

Light regulates many aspects of plant development in addition to phototropism. The affected processes range from seed germination to shoot elongation to the initiation of flowering. Several photoreceptors take part in these and other processes.

Five phytochromes mediate the effects of red and dim blue light. Three or more blue-light receptors, discovered more recently, mediate the effects of higher-intensity blue light.

37.21 Sensitivity of Seeds to Light

In each case, the final exposure reverses the preceding exposure; seeds respond only to the wavelength of the final light exposure.

Phytochromes mediate the effects of red and far-red light

Some seeds will not germinate in darkness, but do so readily after even a brief exposure to light. Blue and red light are highly effective in promoting germination, whereas green light is not.

Of particular importance to plants is the fact that far-red light reverses the effect of a prior exposure to red light. Far-red light is a very deep red, bordering on the limit of human vision and centered on a wavelength of 730 nm; red wavelengths are around 660 nm. If exposed to brief, alternating periods of red and far-red light in close succession, lettuce seeds respond only to the final exposure: If it is red, they germinate; if it is far-red, they remain dormant (Figure 37.21). This reversibility of the effects of red and far-red light regulates many other aspects of plant development, including flowering and seedling growth.

The basis for the red and far-red effects resides in certain bluish photoreceptor proteins called phytochromes. They are blue because they absorb red and far-red light and transmit other light. In the cytosol of plants are two interconvertible forms of phytochromes. Light drives the interconversion of the two forms. The form that absorbs principally red light is called P_r . Upon absorption of a photon of red light, a molecule of P_r is converted into P_{fr} . The P_{fr} form absorbs far-red light; when it does so, it is converted to P_r .

Red light

Phytochrome (P_r) absorbs red light.



Phytochrome (P_{fr}) absorbs far-red light.

Far-red light

EXPERIMENT

^H

Question: How do red and far-red light affect lettuce seed germination?

METHOD

Q Lettuce seeds were exposed to alternate periods of red light Q for 1 minute and far-red light HH for 4 minutes.

o Seeds germinate if the final exposure is to red o ...

Q ...and remain dormant if the final exposure is to far-red QQ.

a

RESULTS

QQ3

R FR RFR RFR KIR FR R FR R FR R FR



Most germinate Few germinate

Most germinate

Few germinate

Conclusion: R and FR reverse each other's effects.

662 CHAPTER THIRTY-SEVEN

P_{tr} has some important biological effects. As we have just seen, one of them is to initiate germination in certain seeds, such as lettuce.

Phytochromes have many effects

Phytochromes help to regulate a seedling's early growth. The radicle, or embryonic root, is the first portion of the seedling to escape the seed coat (see Figure 37.6); the shoot emerges later. When seeds germinate in the dark below the soil surface, a pale and spindly seedling forms, with undeveloped leaves. Such an etiolated plant cannot carry out photosynthesis. The seedling shoot must reach the soil surface and begin photosynthesis before its nutrient reserves are expended and it starves.

Plants have evolved a variety of ways to cope with the problem of germinating underground. Etiolated flowering plants, for example, do not form chlorophyll. They turn green only when exposed to light, thereby conserving the resources needed to make chlorophyll, which would be useless in the dark. An etiolated shoot uses stored resources to elongate rapidly and hasten its arrival at the soil surface, where photosynthesis quickly begins. To break through soil yet protect delicate, underdeveloped leaves, the shoot of an etiolated eu-dicot seedling forms an apical hook (see Figure 37.19).

All of these etiolation phenomena (lack of chlorophyll, rapid shoot elongation, production of an apical hook, delayed leaf expansion) are regulated by the phytochromes. In a seedling that has never been exposed to light, all the phytochrome is in the red-absorbing (P_r) form. Exposure to light converts P_r to P_{fr} (the far-red-absorbing form), and the P_{fr} initiates reversal of the etiolation phenomena: Chlorophyll synthesis begins, shoot elongation slows, the apical hook straightens out, and the leaves start to expand.

There are multiple phytochromes

For years, plant biologists had difficulty accounting for some aspects of phytochrome action. A solution to these problems may lie in the discovery of multiple forms of phytochromes and other photoreceptors. Arabidopsis has five genes that encode different phytochromes, and this diversity has been found throughout the plant kingdom and in algae as well.

The several phytochromes may play differing roles in various phytochrome-controlled responses. Some of them may even play off each other to fine-tune plant growth during the day. Consider, for example, the light spectrum available to a seedling that is growing in the shade of other plants. Because chlorophyll in the leaves above it absorbs the light first, the shaded seedling "sees" a spectrum relatively rich in far-red (and poor in red); the ratio of far-red to red is increased as much as 10-fold to 20-fold in the shade. The interplay among signal transduction pathways initiated by the different phytochromes may lead to an increased rate of stem elongation that tends to bring the leaf into full sunlight.

We do not yet know how the various phytochromes produce their many effects, although it is evident that phy-

tochromes act through the plant's genome. Phytochromes appear to activate one or more G proteins. G proteins are membrane proteins that must bind to guanosine triphosphate (GTP) to exert their effects (see Chapter 15). The phytochrome-activated G proteins may convert GTP into the second messenger cGMP (cyclic guanosine monophosphate) and open channels that admit calcium ions into the cell, where they bind to the protein calmodulin. Both cGMP and the calcium-calmodulin complex can trigger changes leading eventually to the activation of specific genes.

Cryptochromes and phototropin are blue-light receptors

Cryptochromes are yellow photoreceptor pigments that absorb blue and ultraviolet light. They affect some of the same developmental processes, including seedling development and flowering, as do phytochromes. Unlike phytochromes, cryptochromes are present and play important roles in animals as well as plants.

In contrast to phytochromes, cryptochromes are located primarily in the plant nucleus. The exact mechanism of cryptochrome action is not yet known. It may be significant that phytochromes behave like protein kinases, and that cryptochromes can be substrates of such enzymes. It is likely that both classes of photoreceptors participate in protein kinase-based signaling pathways (see Chapter 15).

We began this chapter with a photo of a plant's phototropic response. Later we saw that the study of phototro-

Figure 37.22 A Nonphototropic Mutant

(a) The four etiolated wild-type Arabidopsis seedlings in the top row are demonstrating normal phototropism. (b) These mutant seedlings cannot produce phototropin, the photoreceptor that signals the plant to curve toward light.

PLANT GROWTH REGULATION 663

pism led to the discovery of auxin. But a question remained: What is the photoreceptor for phototropism? Plant scientists working with phototropic mutants of Arabidopsis have recently showed convincing evidence that it is a yellow protein, which they named phototropin. Upon absorbing blue light, phototropin initiates a signal transduction pathway leading to phototropic curvature (Figure 37.22). Still another type of blue-light receptor may be responsible for the light-induced closure of stomata.

Plants respond to light in many ways, and their responses are mediated by interactions of several photoreceptors, as we have seen.

Chapter Summary

Interacting Factors in Plant Development

- ▶ The environment, photoreceptors, hormones, and the plant's genome all play roles in the regulation of plant development.
- ▶ Hormones mediate many developmental phenomena in plants. Each plant hormone plays multiple regulatory roles, affecting several different aspects of development. Interactions among the hormones are often complex. Review Table 37.1
- ▶ Hormones and photoreceptors act through signal transduction pathways.

From Seed to Death: An Overview of Plant Development

- ▶ Cell division, cell expansion, and cell differentiation all contribute to plant development.
- ▶ The dormant seed eventually germinates and forms a growing seedling. Photoreceptors and hormones regulate seedling development, including growth. Review Figures 37.1, 37.2
- ▶ Eventually the plant flowers and forms fruit. Flowering in some plants is controlled by the length of the night. Hormones, probably including a flowering hormone, play roles in plant reproduction. Review Figure 37.3
- ▶ Some plant buds demonstrate winter dormancy. Eventually, all plants senesce and die. Dormancy and senescence are triggered by environmental cues, mediated by photoreceptors and hormones.

Ending Seed Dormancy and Beginning Germination

- ▶ Seed dormancy may be caused by exclusion of water or oxygen from the embryo, mechanical restraint of the embryo, or chemical inhibition of embryo development. In nature, seed dormancy is broken in various ways, including scarification, fire, leaching, and low temperatures.
- ▶ Seed dormancy offers adaptive advantages, such as an increased likelihood of germination in a place and at a time favorable for seedling growth.
- ▶ Seed germination begins with the imbibition of water. Then the embryo mobilizes its reserves to obtain building blocks and energy. The embryos of cereal seeds secrete gibberellins, which cause the aleurone layer to synthesize and secrete digestive enzymes that break down large molecules stored in the endosperm. Review Figure 37.7

Gibberellins: Regulators from Germination to Fruit Growth

- ▶ There are dozens of gibberellins. One, gibberellin A_v regulates stem growth in most plants.
- ▶ Mutant plants that cannot produce normal amounts of gibberellins are dwarfs: Their stems are shorter than wild-type stems.
- ▶ Gibberellins regulate the growth of some fruits and cause bolting in some biennial plants. Review Figure 37.9

Auxin Affects Plant Growth and Form

- ▶ Studies of phototropism led to the discovery and isolation of auxin (indoleacetic acid). In grass seedlings, the photoreceptor for phototropism is in the tip of the coleoptile, and auxin is a messenger from the photoreceptor to the growing region of the coleoptile. Review Figures 37.10, 37.11
- ▶ Auxin transport is polar. Lateral movement of auxin establishes shoot and root responses to light and gravity: phototropism and gravitropism, respectively. Auxin carrier proteins move auxin into and out of cells. Review Figure 37.12
- ▶ Auxin plays roles in root formation, leaf abscission, apical dominance, and parthenocarpic fruit development. Certain synthetic auxins are used as selective herbicides. Review Figures 37.13, 37.14
- ▶ The arrangement of cellulose microfibrils in the plant cell wall limits the rate and direction of cell growth. Auxin increases the plasticity of the cell wall, promoting cell expansion. Part of the auxin response results from the pumping of protons from the cytoplasm into the cell wall, where the lowered pH activates proteins called expansins. Review Figures 37.15, 37.16, 37.17
- ▶ Like all plant hormones, auxin is bound by receptor proteins.
- ▶ Auxin and other plant hormones signal cell differentiation and organ formation.

Cytokinins Are Active from Seed to Senescence

- ▶ Cytokinins are adenine derivatives. Zeatin and isopentenyl adenine are naturally occurring cytokinins, and kinetin is a synthetic cytokinin.
- ▶ First studied as promoters of plant cell division, cytokinins also promote seed germination in some species, inhibit stem

elongation, promote lateral swelling of stems and roots, stimulate the growth of lateral buds, promote the expansion of leaf tissue, and delay leaf senescence.

Ethylene: A Gaseous Hormone That Promotes Senescence

- ▶ A balance between auxin and ethylene controls leaf abscission.
- ▶ Ethylene promotes senescence and fruit ripening.
- ▶ Ethylene causes the formation of a protective apical hook in eudicot seedlings that have not been exposed to light. In stems, it inhibits elongation, promotes lateral swelling, and causes a loss of gravitropic sensitivity.
- ▶ Ethylene acts through a signal transduction pathway that includes two proteins in the plasma membrane and that leads to the expression of genes. Review Figure 37.20

Abscisic Acid: The Stress Hormone

- ▶ Abscisic acid appears to maintain winter dormancy in buds. It prevents seeds from germinating while still attached to the parent plant, and it inhibits stem elongation. Through its effects on stomatal opening, it also regulates gas and water exchange between leaves and the atmosphere.

Hormones in Plant Defenses

- ▶ Oligosaccharins are hormones released by the cell wall in response to an attack by a pathogen. They participate in

664 CHAPTER THIRTY-SEVEN

plant defenses against pathogens, and they interact in complex ways with auxin.

Brassinosteroids: "New" Hormones with Multiple Effects

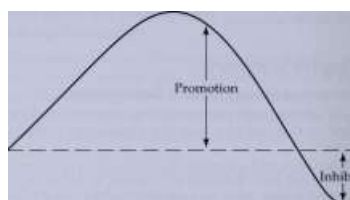
- ▶ There are dozens of brassinosteroids. They affect cell elongation, pollen tube elongation, vascular tissue differentiation, and root elongation.

Light and Photoreceptors

- ▶ Photochromes are bluish proteins found in the cytosol. Each phytochrome exists in two forms, P_r and P_{fr} , that are interconvertible by light. P_r absorbs red light (with a maximum at 660 nm), and P_{fr} absorbs far-red light (730 nm). Review Figure 37.21
- ▶ Photochromes have many effects, including the various manifestations of etiolation.
- ▶ There are five phytochromes. They may play different roles in development, and their signal transduction pathways may interact to mediate the effects of light environments of differing spectral distribution. They mediate the effects of red and low-energy blue light.
- ▶ Cryptochromes, yellow photoreceptor proteins that absorb blue and ultraviolet light, interact with phytochromes in controlling seedling development and floral initiation. Cryptochromes mediate high-energy blue light effects.
- ▶ The signaling pathways for phytochromes and cryptochromes are based on protein kinases.
- ▶ Phototropin, another yellow protein, is the photoreceptor for phototropism.

For Discussion

1. How may it be advantageous for some species to have seeds whose dormancy is broken by fire?
2. Cocklebur fruits contain two seeds each, and the two seeds are kept dormant by two different mechanisms. How may this use of two mechanisms of dormancy be advantageous to cockleburs?
3. Corn stunt virus causes a great reduction in the growth rate of infected corn plants, so the diseased plants take on a dwarfed form. Since their appearance is reminiscent of the genetically dwarfed corn studied by Phinney, you suspect that the virus may inhibit the synthesis of gibberellins by the corn plants. Describe two experiments you might conduct to test this hypothesis, only one of which should require chemical measurement.
4. Whereas relatively low concentrations of auxin promote the elongation of segments cut from young plant stems, higher concentrations generally inhibit growth, as shown in the figure.



Inhibition

Auxin concentration

In some plants, the inhibitory effects of high auxin concentrations appear to be secondary: High auxin concentrations cause the synthesis of ethylene, which is what causes the growth inhibition. Silver thiosulfate inhibits ethylene action. How do you think the addition of silver thiosulfate to the solutions in which the stem segments grew would affect the appearance of the above graph?

Some etiolated seedlings develop hairs on their epidermis when exposed to dim light. Describe an experiment to test the hypothesis that a phytochrome is the photoreceptor for this effect.



Reproduction in Flowering Plants



Biologists have known for more than 60 years that the leaves of some plants, such as the cocklebur, contain built-in timers that measure the length of every night. When the night is of the appropriate length, the leaves—even a single leaf on a plant stripped of all other leaves—send a signal to other parts of the plant, telling them to form flowers. The evidence for this signal is substantial and convincing, yet nobody has been able to isolate and identify it.

After years of frustration, we may soon solve this mystery. The probable key lies in recent discoveries, described earlier in this book, about the functioning of plasmodesmata, the minute passageways connecting adjacent plant cells. Studies using mutant plants may allow scientists to identify the signal and learn how it triggers flowering. This knowledge will be a major advance in our understanding of reproduction in plants.

Why do plants expend energy and resources to produce flowers? The answer is simple: Flowers are sexual reproductive structures, and reproduction is one of the most important events in a plant's—or any organism's—life.

In this chapter we look at several aspects of plant reproduction, including some that are still not well understood. We contrast sexual and asexual reproduction, and we consider sexual reproduction in detail. In doing so, we look at angiosperm gametophytes, pollination, double fertilization, embryonic development, and the roles of fruits in seed dispersal. The transition to the flowering state is a key event in plant development, and we'll see how changing seasons trigger flowering in some plants—and speculate on the existence of a flowering hormone. We conclude the chapter with an examination of asexual reproduction in nature and in agriculture.

Many Ways to Reproduce

Plants have many ways of reproducing themselves—and with humans helping, there are even more ways. Flowers contain the sex organs of plants; it is thus no surprise that almost all flowering plants reproduce sexually. But many reproduce asexually as well; some even reproduce asexually most of the time. What are the advantages and disadvantages

Where It All Began

Some of the best early evidence for a flowering hormone came from studies of cockleburs (*Xanthium* sp.).

What are the advantages and disadvantages of these two kinds of reproduction? The answers to this question involve genetic recombination. As we have seen, sexual reproduction produces new genetic combinations and diverse phenotypes. Asexual reproduction, in contrast, produces a clone of genetically identical individuals.

Both sexual and asexual reproduction are important in agriculture. Many important annual crops are grown from seeds, which are the products of sexual reproduction. Seed-grown crops include wheat, rice, millet, and corn—the great grain crops, all of which are grasses—as well as plants in other families, such as soybeans and safflower. Other crops are produced asexually from grafts, or by other asexual means.

Orange trees, which have been under cultivation for centuries, can be grown from seed—except for one type, the navel orange. This plant apparently arose only once in history. Early in the nineteenth century, on a plantation on the Brazilian coast, one seed gave rise to one tree that had aberrant flowers. Parts of the flowers aborted, and seedless



666 CHAPTER THIRTY-EIGHT

fruits formed. Every navel orange in the world comes from a navel orange tree derived asexually from that original Brazilian tree. Asexual reproduction is the only way of propagating this plant.

Unlike navel oranges, strawberries need not be propagated asexually, because they are capable of forming seeds. Nonetheless, asexual propagation of strawberries is common because vast numbers of plants that are genetically identical to a particularly desirable plant can be produced in this way.

We will treat asexual reproduction in greater detail at the end of this chapter. We begin, however, by considering sexual reproduction.

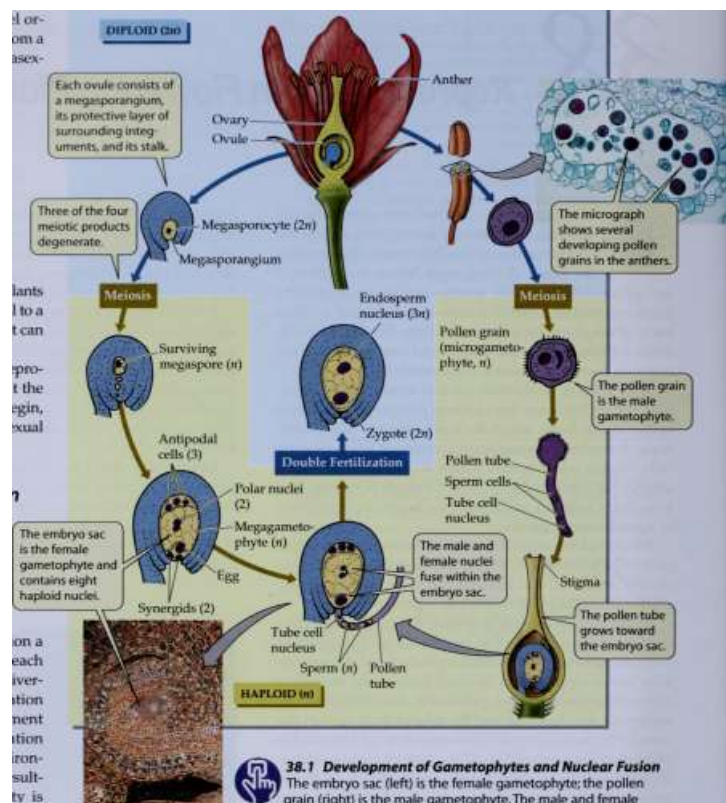
Sexual Reproduction

Sexual reproduction provides genetic diversity through recombination (see Chapter 9). Meiosis and mating shuffle genes into new combinations, giving a population a variety of genotypes in each generation. This genetic diversity may serve the population well as the environment changes or as the population expands into new environments. The adaptability resulting from genetic diversity is the major advantage of sexual reproduction over asexual reproduction.

DIPLOID (2n) I

Each ovule consists of a megasporangium, its protective layer of surrounding integuments, and its stalk.

Three of the four meiotic products degenerate.



'«&§&

38.1 Development of Gametophytes and Nuclear Fusion

The embryo sac (left) is the female gametophyte; the pollen grain (right) is the male gametophyte. The male and female nuclei meet and fuse within the embryo sac.

The flower is an angiosperm's device for sexual reproduction

A complete flower consists of four groups of organs that are modified leaves: the carpels, stamens, petals, and sepals (see Figure 29.6 for review). The carpels and stamens are, respectively, the female and male sex organs. A pistil is a structure composed of one or more carpels. The base of the pistil, called the ovary, contains one or more ovules, each of which contains a megasporangium. The stalk of the pistil is the style, and the end of that stalk is the stigma. Each stamen is composed of a filament bearing a two-lobed anther, which consists of four microsporangia fused together.

The petals and sepals of many flowers are arranged in whorls (circles) around the carpels and stamens. Together, the petals constitute the corolla. Below them, the sepals constitute the calyx. The petals are often colored; the sepals are often green and photosynthetic. All the parts of the flower are borne on a stem tip, the receptacle.

Flowering plants have microscopic gametophytes

Before reading this section, you may wish to review the section in Chapter 28 entitled "Life cycles of plants feature alternation of generations." The concept of alternation of generations is central to an understanding of plant reproduction.

In plants, the sporophyte generation produces flowers. The flowers produce spores, which develop into tiny gametophytes. The flower is more than just a place where the egg and sperm are eventually found—it is also the place where the alternate generation resides.

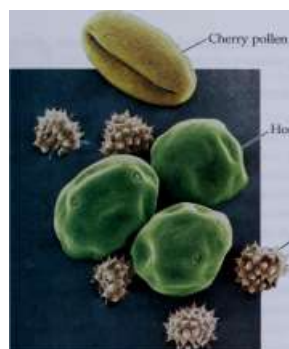
The gametophytes—the gamete-producing generation— of flowering plants develop from haploid spores in sporangia within the flower (Figure 38.1).

- Female gametophytes (megagametophytes), which are called embryo sacs, develop in megasporangia.
- Male gametophytes (microgametophytes), which are called pollen grains, develop in microsporangia.

Within the ovule, a megasporocyte—a cell within the megasporangium—divides meiotically to produce four haploid megaspores. All but one of these megaspores then degenerate. The surviving megaspore undergoes mitotic divisions, usually producing eight haploid nuclei, all initially contained within a single cell—three nuclei at one end, three at the other, and two in the middle. Subsequent cell wall formation leads to an elliptical, seven-celled megagametophyte with a total of eight nuclei.

- At one end of the elliptical megagametophyte are three tiny cells: the egg and two cells called synergids. The egg is the female gamete, and the synergids participate indirectly in fertilization.
- At the opposite end of the megagametophyte are three antipodal cells, which eventually degenerate.
- In the large central cell are two polar nuclei.

Cherry pollen



38.2 A Pollen Grain Sampler

Each species' pollen has a characteristic size, shape, and cell wall formation.

Hornbeam pollen

Daisy pollen

100 urn

Pollen is transferred by the direct contact of anther and stigma within the same flower, resulting in self-fertilization. Wind is the vehicle for pollen transport in many species. Wind-pollinated flowers have sticky or featherlike stigmas, and they produce pollen grains in great numbers (Figure 38.3). Some aquatic angiosperms are pollinated by water action, with water carrying

pollen grains from plant to

The embryo sac is the entire seven-celled, eight-nucleus structure. (Follow the arrows down the left-hand side of Figure 38.1 to review the development of the embryo sac.)

The male gametophyte, or pollen grain, consists of fewer cells than the female gametophyte. The development of a pollen grain begins when a microsporocyte within the anther divides meiotically. Each resulting haploid microspore normally undergoes one mitotic division within the spore wall before the anthers open and release these two-celled pollen grains. Further development of the pollen grain, which we will describe shortly, is delayed until the pollen arrives at a stigma. In angiosperms, the transfer of pollen from the anther to the stigma is referred to as pollination.

Pollination enables fertilization in the absence of liquid water

Gymnosperms and angiosperms evolved independence from liquid water as a medium for gamete travel and fertilization—a freedom not shared by other plant groups. The male gametes of gymnosperms and angiosperms travel within pollen grains (Figure 38.2). But how do angiosperm pollen grains travel from an anther to a stigma?

Many different mechanisms have evolved for pollen transport. In some plants, such as peas and their relatives, pollination is accomplished before the flower bud opens.



Corylus cornuta

38.3 Wind Pollination

The numerous anthers on these inflorescences (groups of flowers) of a hazelnut tree all point away from the stalk and stand free of the plant, promoting dispersal of the pollen by wind.

668 CHAPTER THIRTY-EIGHT

plant. Animals, including insects, birds, and bats, carry pollen among the flowers of many plants.

Some plants practice "mate selection"

In our discussion of Mendel's work (see Chapter 10), we saw that some plants can reproduce sexually either by cross-pollinating or by self-pollinating. Many plants demonstrate self-incompatibility; that is, they reject pollen from their own flowers. This rejection promotes genetic variation and limits inbreeding. A single gene, the S gene, is responsible for self-incompatibility. The S gene has dozens of alleles. A pollen grain is haploid and possesses a single S allele; the recipient stigma is diploid. In self-incompatible plants, pollen fails to germinate, or develops abnormally, on a stigma that possesses the same S allele (Figure 38.4).

The stigma plays an important role in "mate selection" by flowering plants. The stigmas of wind-pollinated plants are exposed to the pollen of many other species as well as their own, and even the flowers of plants with coevolved, specific animal pollinators may receive pollen from other plant species. Pollen from the same species binds strongly to the stigma due to cell-cell signaling by the cell wall of pollen grains of the same species. In contrast, foreign pollen falls off readily, without germinating.

A pollen tube delivers male cells to the embryo sac

When a pollen grain lands on the stigma of a compatible pistil, a pollen tube develops from the pollen grain (Figure 38.5). The pollen tube either digests its way through the spongy tissue of the style or, if the style is hollow, grows downward on the inner surface of this female organ. The pollen tube grows millimeters or even centimeters in the process.

Pollen grain

Pollen tube

Stigma

Pollen

Pollen

Stigma

Style

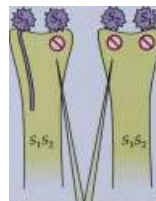
Pollen

tube

Ovary



S₃ and S₄ pollen are compatible with S₁S₂ stigma.



...but S₁ and S₂ pollen do not germinate. They are self-incompatible.

38.4 Self-Incompatibility

Pollen grains do not germinate normally if their S allele matches one of the S alleles of the stigma. Thus, the egg cannot be fertilized by a sperm from the same plant.



38.5 Pollen Tubes Begin to Grow

Pollen grains have landed on hairlike structures on the stigma of an Arabidopsis flower. Pollen tubes have started to form.

The rapid growth of the pollen tube requires calcium ions, taken up at the growing tip of the tube, as well as cell adhesion proteins. The downward growth of the pollen tube is guided by a long-distance signal from the ovule.

Angiosperms perform double fertilization

The pollen grain consists of two cells. The larger tube cell encloses the much smaller generative cell (Figure 38.6). Guided by the tube cell nucleus, the pollen tube eventually grows through megasporangial tissue and reaches the embryo sac. The generative cell meanwhile has undergone one mitotic division and cytokinesis to produce two sperm cells.

Both of the sperm cells enter the embryo sac, where they are released into the cytoplasm of one of the synergids. This synergid degenerates, releasing the sperm cells. Each sperm cell then fuses with a different cell of the embryo sac. One sperm cell fuses with the egg cell, producing the diploid zygote. The other fuses with the central cell, and that sperm cell nucleus and the two polar nuclei unite to form a triploid (3n) nucleus. While the zygote nucleus begins division to form the new sporophyte embryo, the triploid nucleus undergoes rapid mitosis to form a specialized nutritive tissue, the endosperm. The antipodal cells and the remaining synergid eventually degenerate, as does the pollen tube nucleus.

The fusion of a sperm cell nucleus with polar nuclei to form endosperm takes place only in angiosperms. This and the possession of flowers are the two most definitive characteristics shared by all angiosperms.

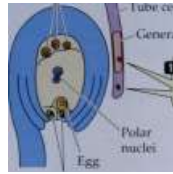
Embryos develop within seeds

Shortly after fertilization, highly coordinated growth and development of embryo, endosperm, integuments, and carpel

ensues. The integuments develop into a double-layered seed coat, and the carpel ultimately becomes the wall of the fruit that encloses the seed.

Three antipodal cells

- Tube cell

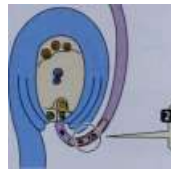


Generative cell

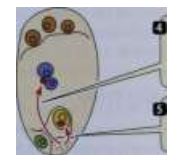
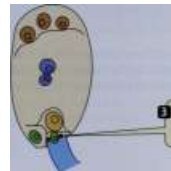
| Initially the pollen tube contains two haploid cells, the generative cell and the tube cell.

Polar nuclei

Egg Synergids



The generative cell divides mitotically, producing two haploid sperm cells.



The sperm cells enter the cytoplasm of a synergid.

| The synergid breaks down; one sperm nucleus unites with the two polar nuclei, forming the first cell of the $3n$ endosperm generation.

The other sperm nucleus fertilizes the egg, forming the zygote, the first cell of the $2n$ sporophyte generation.



t 38.6 Pollen Nuclei and Double Fertilization

The sperm nuclei contribute to the formation of the diploid zygote and the triploid endosperm. Double fertilization is a characteristic feature of angiosperm reproduction.

The first step in the normal formation of the embryo is a mitotic division of the zygote—the fertilized egg—giving rise to two daughter cells. Even at this stage, the two cells face different fates. An asymmetrical (uneven) distribution of cytoplasm within the zygote causes one end to produce the embryo proper and the other end to produce a supporting structure, the suspensor (Figure 38.7). The suspensor pushes the embryo against or into the endosperm, and provides one route by which nutrients enter the embryo.

With the asymmetrical division of the zygote, polarity has been established, as has the longitudinal axis of the new plant. A filamentous suspensor and a globular embryo are distinguishable after just four mitotic divisions. The suspensor soon ceases to elongate. In the embryo, the primary meristems form. As development continues, the first organs take form within the embryo.

In eudicots (monocots are somewhat different), the initially globular embryo takes on a characteristic heart-stage form as the cotyledons start to grow. Further elongation of the cotyledons and of the main axis of the embryo gives rise to what is called the torpedo stage (see Figure 38.7), during which some of the internal tissues begin to differentiate. The elongating region below the cotyledons is the hypocotyl. At the top of the hypocotyl, between the cotyledons, is the shoot apex; at the other end is the root apex. Each of these apical regions contains an apical meristem whose dividing cells will give rise to the organs of

the mature plant.

Large amounts of nutrients are moved in from other parts of the plant, and the endosperm accumulates starch,

Endosperm nucleus

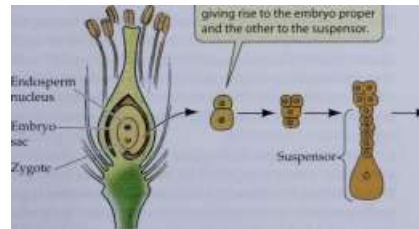
The zygote nucleus divides mitotically, one daughter cell giving rise to the embryo proper and the other to the suspensor.

Zygote



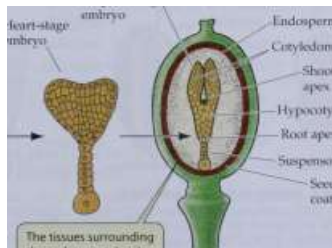
t 38.7 Early Development of a Eudicot

The embryo develops through intermediate stages, including a characteristic heart-shaped form, to reach the torpedo stage.



Heart-stage embryo

Torpedo-stage embryo



The tissues surrounding the embryo sac develop into the seed coat.

670 CHAPTER THIRTY-EIGHT

lipids, and proteins. In many species, the cotyledons absorb the nutrient reserves from the surrounding endosperm and grow very large in relation to the rest of the embryo (Figure 38.8rt). In others, the cotyledons remain thin (Figure 38. 8b); they draw on the reserves in the endosperm as needed when the seed germinates.

In the late stages of embryonic development, the seed loses water—sometimes as much as 95 percent of its original water content. In its dried state, the embryo is incapable of further development. It remains in this quiescent state until the conditions are right for germination. (Recall from Chapter 37 that a necessary first step in seed germination is the massive imbibition of water.)

Some fruits assist in seed dispersal

After fertilization, the ovary wall of a flowering plant—together with its seeds—develops into a fruit. A fruit may consist of only the mature ovary and its seeds, or it may include other parts of the flower or structures that are closely related to it. Some major variations on this theme are illustrated in Figure 29.11, which shows only fleshy, edible fruits. Many other fruits are dry or inedible.

Some fruits help disperse seeds over substantial distances. Various trees, including ash, elm, maple, and tree of heaven, produce a dry, winged fruit that may be blown some distance from the parent tree by the wind (Figure 38.9a). Water disperses some fruits; coconuts have been spread in this way from island to island in the Pacific (Figure 38.9b). Still other fruits travel by hitching rides with animals—either inside or outside them. Fleshy fruits such as berries provide food for mammals or birds; their seeds travel safely through the animal's digestive tract and are deposited some distance from the parent plant.

We have traced the sexual life cycle from the flower to the fruit to the dispersal of seeds. We discussed seed germination and vegetative development of the seedling in

In some eudicots, the cotyledons absorb much of the endosperm and fill most of the seed.

In other eudicots, the endosperm remains separate and the cotyledons remain thin.

In monocots, the single cotyledon is pressed against the endosperm.



Seed coat Cotyledon Shoot apex



Seed coat



Cotyledon Shoot apex —

Root apex ^^^Y\J

Cotyledon

Endosperm- ' ^j^P Endosperm

(a) Kidney bean (b) Castor bean (c) Corn

38.8 Variety in Angiosperm Seeds

In some seeds, such as kidney beans (a), the nutrient reserves of the endosperm are absorbed by the cotyledons at the seed stage. In others, such as castor beans (b) and corn (c), the reserves in the endosperm will be drawn on throughout the course of development.



(b)

38.9 Dispersal of Fruits

(a) A samara is a winged fruit characteristic of the maple family, (b) A coconut seed germinates where it washed ashore on a beach in the South Pacific.

Chapter 37. Now let's complete the sexual life cycle by considering the transition from the vegetative to the flowering state, and how this transition is regulated.

The Transition to the Flowering State

Flowering may terminate, interrupt, or accompany vegetative growth. The transition to the flowering state marks the end of vegetative growth for some plants. If we view a plant as something produced by a seed for the purpose of bearing more seeds, then the act of flowering is one of the supreme events in a plant's life.

Apical meristems can become inflorescence meristems

The first visible sign of the transition to the flowering state may be a change in one or more apical meristems in the shoot system. During vegetative growth, an apical meristem continually produces leaves, lateral buds, and internodes (regions of stem between the nodes where leaves and buds form: Figure 38.10f). This unrestricted growth is indeterminate (see Chapter 34).

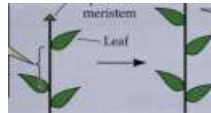
Flowers may appear singly or in an orderly cluster that constitutes a structure called an inflorescence. If a vegetative meristem becomes an inflorescence meristem, it generally produces several other structures: smaller leafy structures called bracts, as well as new meristems in the angles between the bracts and the internodes (Figure 38.10b).

(«)

An internode is a section of stem between two nodes (places where leaves or buds form).

/Vegetative apical meristem

C7



REPRODUCTION IN FLOWERING PLANTS 671

f

A vegetatively growing apical meristem continues to produce leaves and internodes.

(b)

Inflorescence meristems give rise to floral meristems, bracts, and even inflorescence meristems.

O'

<3

, Inflorescence meristem

o

^

'Floral meristem

A bract is a modified, usually reduced leaflike structure.

(7

(c)

A floral meristem

gives rise to a flower.



Floral meristem

\\/



- Carpel

Stamen

Petal

Sepal

Modified leaflike structures of the flower

38.10 Flowering and the Apical Meristem

A vegetative apical meristem (a) grows without producing flowers. Once the transition to the flowering state is made, inflorescence meristems (b) give rise to bracts and to floral meristems (c), which become the flowers.

These new meristems may also be inflorescence meristems, or they may be floral meristems, which give rise to the flowers themselves.

Each floral meristem typically produces four consecutive whorls of organs—the sepals, petals, stamens, and carpels—separated by very short internodes, keeping the flower compact (Figure 38.10c). In contrast to vegetative meristems and some inflorescence meristems, floral meristems are responsible for determinate growth—the limited growth of the flower to a particular size and form.

A cascade of gene expression leads to flowering

How do apical meristems become inflorescence meristems, and how do inflorescence meristems give rise to floral meristems? How does a floral meristem give rise, in short order, to four different organs? How does each flower come to have the correct number of each of the floral organs? Numerous genes collaborate to produce these results. We'll refer here to some of the genes whose actions have been most thoroughly understood in *Arabidopsis* and snapdragons.

In order for an inflorescence meristem to give rise to a floral meristem, a group of floral meristem identity genes must be expressed. Expression of these genes initiates a cascade of further gene expression. Another set of genes, part of this cascade, participates in pattern formation—the spatial organization of the whorls of organs, which are still to be

determined. These genes, in turn, trigger the expression of a group of organ identity genes that work in concert to specify the successive whorls (see Figure 16.11). They are homeotic genes, and their products are transcription factors that mediate the expression of still further genes.

Now that we have seen how flowering occurs, we will consider how the transition from the vegetative to the flowering state is initiated.

Photoperiodic Control of Flowering

The life cycles of flowering plants fall into three categories: annual, biennial, and perennial. Annuals, such as many food crops, complete their life cycle (seed to flower) in less than a year. Biennials, such as carrots and cabbage, grow for all or part of one year and live on into a second year, during which they flower, form seeds, and die. Perennials, such as oak trees, live for a few to many years, during which both growth and flowering occur. What control systems give rise to these and other differences in flowering behavior?

In 1920, W. W. Garner and H. A. Allard of the U.S. Department of Agriculture studied the behavior of a newly discovered mutant tobacco plant. The mutant, named 'Maryland Mammoth,' had large leaves and exceptional height. When the other plants in the field flowered, the 'Maryland Mammoth' continued to grow. Garner and Allard took cuttings of the 'Maryland Mammoth' into their greenhouse, and the plants that grew from the cuttings finally flowered in December.

Garner and Allard guessed that this pattern had something to do with the seasons. They tested several likely seasonal variables, such as temperature, but the key variable proved to be the length of the day (as they saw it). By moving plants between light and dark rooms at different times to vary the day length artificially, they were able to establish a direct link between flowering and day length. (We now know that the key variable is the length of the night, rather than the day, but Garner and Allard did not make that distinction.)

The 'Maryland Mammoth' plants did not flower if the light period was longer than 14 hours each day, but flowering commenced after the days became shorter than 14 hours. Thus, the critical day length for 'Maryland Mammoth' tobacco is 14 hours (Figure 38.11). This phenomenon of control by the length of day or night is called photoperiodism.

There are short-day, long-day, and day-neutral plants

Poinsettias, chrysanthemums, and 'Maryland Mammoth' tobacco are short-day plants (SDP's), which flower only when the day is shorter than a critical maximum. Spinach and clover are examples of long-day plants (LDP's), which flower only when the day is longer than a critical minimum.

672 CHAPTER THIRTY-EIGHT

Maryland Mammoth' tobacco flowers only when days are shorter than 14 hours; that is, its critical day length is 14 hours.

14 hours Light | Dark



'Maryland

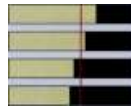
Mammoth' tobacco

(short-day plant)



Henbane flowers only when days are longer than 14 hours, its critical day length.

14 hours Light | Dark



Henbane,

Hyoscyamus niger

(long-day plant)



Long days;

plant remains vegetative

Short days; plant flowers

Long days; plant flowers

Short days;

plant remains

vegetative

38.11 Day Length and Flowering

By artificially varying the length of the day, Garner and Allard showed that the flowering of Maryland Mammoth tobacco is initiated when the days become shorter than a critical length. Maryland Mammoth tobacco is thus called a short-day plant. Henbane, a long-day plant, shows an inverse pattern of flowering.

Generally, LDP's are triggered to flower in midsummer and SDP's in late summer, or sometimes in the spring.

Some plants require photoperiodic signals that are more complex than just short or long days in order to flower. One group, the short-long-day plants, must first experience short days and then long ones. Accordingly, white clover and other short-long-day plants flower during the long days before midsummer. Another group, the long-short-day plants, cannot flower until the long days of summer have been followed by shorter ones, so they bloom only in the fall. Kalanchoe, seen in Figure 38.17b, is a long-short-day plant.

Other effects besides flowering are also under photoperiodic control. We have learned, for example, that short days trigger the onset of winter dormancy in plants. (Animals, too, show a variety of photoperiodic behaviors.)

The flowering of some angiosperms, such as corn and tomatoes, is not photoperiodic. In fact, there are more of these day-neutral plants than there are short-day and long-day plants. Some plants are photoperiodically sensitive only when young and become day-neutral as they grow older. Others require specific combinations of day length and other factors—especially temperature—to flower.

The length of the night determines whether a plant will flower

The terms "short-day plant" and "long-day plant" became entrenched before scientists learned that plants actually measure the length of the night, or of a period of darkness, rather than the length of the day. This fact was demonstrated by Karl

Hamner of the University of California at Los Angeles and James Bonner of the California Institute of Technology (Figure 38.12).

Working with cocklebur, an SDP, Hamner and Bonner ran a series of experiments using two sets of conditions:

- ▶ The light period was kept constant—either shorter or longer than the critical day length—and the dark period was varied.
- ▶ The dark period was kept constant and the light period was varied.

The plants flowered under all treatments in which the dark period exceeded 9 hours, regardless of the length of the light period. Thus it is the length of the night that matters; for cocklebur, the critical night length is about 9 hours. Thus, it would be more accurate to call cocklebur a "long-night plant" than a short-day plant.

EXPERIMENT

Question: Do short-day plants measure the length of day or night?

METHOD

Plants were moved between light and dark rooms for specified numbers of hours.

RESULTS

Light constant/Darkness varied

16

16

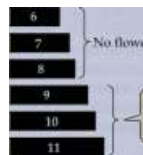
> ■ No flowering

16

16

16

16

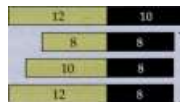


Only plants given > 9 or more hours of dark flowered.

Light varied/8 or 10 hours of darkness

10

Only plants given 10 hours of dark flowered.



> ■ No flowering

Time (hours)

Conclusion: Short-day plants measure the length of night and could more accurately be called long-night plants.

38.12 Night Length and Flowering

The length of the dark period, not the length of the light period, determines flowering.

EXPERIMENT A

Question: What happens if you interrupt a long night or day?

Short-day plants

No flowering

No flowering

No flowering

Long-day Experimental conditions plants

Flowering

Flowering

No flowering



Flowering

Conclusion: Photoperiodic plants measure the length of the night, not the day. Interrupting a long night with a brief period of light inhibits flowering. Long-day plants flower when the night is short, but interrupting their long day has no effect.

38.13 The Effect of Interrupted Days and Nights

(a) Experiments suggest that plants are able to measure the length of a continuous dark period and use this information to trigger flowering, (b) Phytochromes seem to be involved in the photoperiodic timing mechanism.

the dark period by light, however, completely nullified the effect of a long night (Figure 38.13fl). An SDP flowered only if the long nights were uninterrupted. An LDP experiencing long nights flowered if those nights were broken by exposure to light. Thus a plant must have a timing mechanism that measures the length of a continuous dark period. Despite much study, the nature of this timing mechanism is still unknown.

Phytochromes and blue-light receptors, which affect several aspects of plant development (see Chapter 37), also participate in the photoperiodic timing mechanism. In the interrupted-night experiments, the most effective wavelengths of light were in the red range (Figure 38.13i>), and the effect of a red-light interruption of the night could be fully reversed by a subsequent exposure to far-red light. It was once thought that the timing mechanism might simply be the slow conversion of phytochrome during the night from the Pfr form—produced during the light hours—to the Pr form. But this suggestion is inconsistent with most of the experimental observations and must be wrong. Phytochrome must be only a photoreceptor. The timekeeping role must be played by a biological clock.

Circadian rhythms are maintained by a biological clock

It is abundantly clear that organisms have some way of measuring time, and that they are well adapted to the 24-hour day-night cycle of our planet. Some sort of biological clock resides within the cells of all eukaryotes. The major outward manifestations of this clock are known as circadian rhythms (from the Latin *circa*, "about," and *dies*, "day").

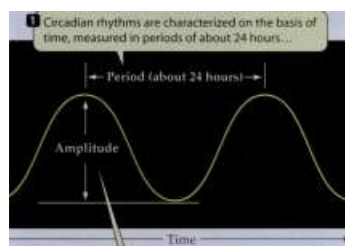
We can characterize circadian rhythms, as well as other regular biological cycles, in two ways: The period is the length of one cycle, and the amplitude is the magnitude of the change over the course of a cycle (Figure 38.14).

In cocklebur, a single long night is enough of a photoperiodic stimulus to trigger full flowering some days later, even if the intervening nights are short ones. Most plants are less sensitive than cocklebur, requiring from two to many nights of appropriate length to induce flowering. For some plants, a single shorter night in a series of long ones, even one day before flowering would have commenced, inhibits flowering.

Hamner and Bonner showed that plants measure the length of the night using another method as well. They grew SDP's and LDP's under a variety of light conditions. Under some conditions, the dark period was interrupted by a brief exposure to light; in others, the light period was interrupted briefly by darkness. Interruptions of the light period by darkness had no effect on the flowering of either short-day or long-day plants. Even a brief interruption of

Effect

Circadian rhythms are characterized on the basis of time, measured in periods of about 24 hours...



?...and on the basis of the magnitude of the rhythmic effect, measured by the cycle's amplitude.

38.14 Features of Circadian Rhythms

The circadian rhythms of plants, like those of other organisms, can be characterized in two ways.

674 CHAPTER THIRTY-EIGHT

Circadian rhythms of protists, animals, fungi, and plants have been found to share some important characteristics:

- ▶ The period is remarkably insensitive to temperature, although lowering the temperature may drastically reduce the amplitude of the fluctuation.
- ▶ Circadian rhythms are highly persistent; they continue even in an environment in which there is no alternation of light and dark.
- ▶ Circadian rhythms can be entrained, within limits, by light-dark cycles that differ from 24 hours. That is, the period an organism expresses can be made to coincide with that of the light-dark regime.
- ▶ A brief exposure to light can shift the rhythm—it can cause a phase shift.

Plants provide innumerable examples of approximately 24-hour cycles. The leaflets of a plant such as clover or the tropical tree *Albizia* normally hang down and fold at night and rise and expand during the day. Flowers of many plants show similar "sleep movements," closing at night and opening during the day. They continue to open and close on an approximately 24-hour cycle even when the light and dark periods are experimentally modified (Figure 38.15).

The period of circadian rhythms in nature is approximately 24 hours. If an *Albizia* tree, for example, were to be

EXPERIMENT

Question: Do plants exhibit circadian rhythms?

METHOD

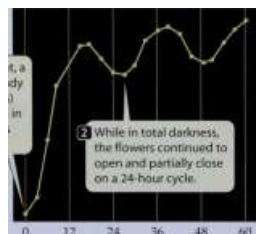
Put *Kalandwe* in continuous darkness. Time the opening and closing of their flowers.

RESULTS

Flower open

At midnight, a plant (already in darkness) was placed in continuous darkness.

Flower closed



12 24 36 48 60

Hours in total darkness

Conclusion: *Kalanchoe* flowers open and close in response to a biological clock even in the absence of external light/dark cues.

38.15 Plants Can Measure Time

Even when *Kalanchoe* is placed in continuous darkness, the opening and closing of its flowers continues to exhibit a circadian rhythm.

placed under electric light on a day-night cycle totaling exactly 24 hours, the rhythm expressed would show a period of exactly 24 hours. However, if an experimenter used a day-night cycle of, say, 22 hours, then over time the rhythm would change—it would be entrained to a 22-hour period.

If an organism is maintained under constant darkness, with its circadian rhythm being expressed on the approximately 24-hour period, a brief exposure to light can cause a phase shift—that is, it can make the next peak of activity appear either later or earlier than expected, depending on when the exposure is given. Moreover, the organism does not then return to its old schedule if it remains in darkness. If the first peak is delayed by 6 hours, the subsequent peaks are all 6 hours late. Such phase shifts are permanent—until the organism receives more exposures to light.

Phytochromes and blue-light receptors are known to affect the period of the biological clock, with the different pigments reporting on different wavelengths and intensities of light. Perhaps this diversity of photoreceptors is an adaptation to the changes in the light environment that a plant experiences.

There is now ample evidence that the photoperiodic behavior of plants is based on the interaction of night length with the

biological clock. But how the clock is coupled with flowering remains unclear.

Is there a flowering hormone?

Is the timing device for flowering located in a particular part of an angiosperm, or are all parts able to sense the length of the night? This question was resolved by "blindfolding" different parts of the plant.

It quickly became apparent that each leaf is capable of timing the night. If a short-day plant is kept under a regime of short nights and long days, but a leaf is covered so as to give it the needed long nights, the plant will flower (Experiment A in Figure 38.16). This type of experiment works best if only one leaf is left on the plant. If one leaf is given a photoperiodic treatment conducive to flowering—an inductive treatment—other leaves kept under noninductive conditions will tend to inhibit flowering.

Although it is the leaves that sense an inductive dark period, the flowers form elsewhere on the plant. Thus a message must be sent from the leaf to the site of flower formation. Three lines of evidence suggest that this message is a chemical substance—a flowering hormone.

► If a photoperiodically induced leaf is removed from the plant shortly after the inductive dark period, the plant does not flower. If, however, the induced leaf remains attached to the plant for several hours, the plant flowers. This result suggests that something must be synthesized in the leaf in response to the inductive dark period, then move out of the leaf to induce flowering.

► If two cocklebur plants are grafted together, and if one plant is given inductive long nights and its graft partner is given noninductive short nights, both plants flower (Experiment B in Figure 38.16).

EXPERIMENT A

Question: What part of the plant measures the dark period?

Cocklebur, a short-day plant, will not flower if kept under long days and short nights.



If even one leaf is masked for part of the day, however—thus shifting that leaf to short days and long nights—the plant will flower; note the burrs.

Burrs

Single leaf masked on long-night/ short-day regimen

Plant does not flower

Plant flowers

EXPERIMENT B

Question: How stable is the flowering hormone?

Five cocklebur plants are grafted together and kept under long days and short nights, with most leaves removed.

If a leaf on a plant at one end of the chain is subjected to long nights, all of the plants will flower.

Leaf induced by long nights/ short days

Branching

experimentally

induced

Conclusion: The leaves measure the dark period. Therefore, some message must move from the induced leaf to the flowering parts of the plant.

38.16 Evidence for a Flowering Hormone

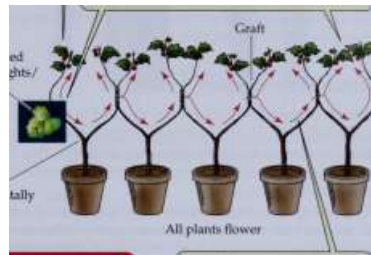
If even a single leaf is exposed to inductive conditions, a "message" travels to the entire plant (and even to other plants, in grafting experiments), inducing it to flower.

► In at least one species, if an induced leaf from one plant is grafted onto another, noninduced plant, the host plant flowers.

Jan A. D. Zeevaart, a plant physiologist now at Michigan State University, performed this last experiment. He exposed a single leaf of the SDP *Perilla* to a short-day/long-night regime, inducing the plant to flower. Then he detached this leaf and grafted it onto another, noninduced, *Perilla* plant—which responded by flowering. The same leaf grafted onto successive hosts caused each of them to flower in turn. As long as 3 months after the leaf was exposed to the short-day/long-night regime, it could still cause plants to flower.

Experiments such as Zeevaart's suggest that the photoperiodic induction of a leaf causes a more or less permanent change in it, inducing it to start and continue producing a flowering hormone that is transported to other parts of the plant, switching those target parts to the reproductive state. So reasonable is this idea that biologists have named this hormone florigen, even though, after decades of active searching, it has not been isolated and characterized.

An elegant experiment suggested that the florigen of short-day plants is identical to that of long-day plants, even though SDP's produce it only under long nights and LDP's only under short nights. An SDP and an LDP were grafted together, and both flowered, as long as the photoperiodic



Conclusion: The very stable flowering message can even travel across multiple grafts.

Arrows indicate the routes of the hypothetical flowering hormone from the induced leaf.

conditions were inductive for one of the partners. Either the SDP or the LDP could be the one induced, but both would always flower. These results suggest that a flowering hormone—the elusive florigen—was being transferred from one plant to the other.

The direct demonstration of florigen activity remains a cherished goal of plant physiologists. For a long time it was thought that florigen could be neither a protein nor an RNA because these molecules were too large to pass from one living plant cell to another. However, we now know that such macromolecules can be transferred by way of plasmodesmata, and biologists are reexamining the possibility that an RNA or a protein is the long-sought florigen.

Vernalization and Flowering

In both wheat and rye, we distinguish two categories of flowering behavior. Spring wheat, for example, is sown in the spring and flowers in the same year. It is an annual plant. Winter wheat is biennial and must be sown in the fall; it flowers in the following summer. If winter wheat is not exposed to cold after its first year, it will not flower normally the next year. The implications of this finding were of great agricultural interest in Russia because winter wheat is a better producer than spring wheat, but it cannot be grown in some parts of Russia because the winters there are too cold for its survival.

Several studies performed in Russia during the early 1900s demonstrated that if seeds of winter wheat were pre-moistened and prechilled, they could be sown in the spring, and would develop and flower normally the same

676 CHAPTER THIRTY-EIGHT

year. Thus, high-yielding winter wheat could be grown even in previously hostile regions. This induction of flowering by low temperatures is called vernalization.

Vernalization may require as many as 50 days of low temperatures (in the range from about -2° to $+12^{\circ}\text{C}$). Some plant species require both vernalization and long days to flower. There is a long wait from the cold days of winter to the long days of summer, but because the vernalized state easily lasts at least 200 days, these plants do flower when they experience the appropriate night length.

Asexual Reproduction

Although sexual reproduction takes up most of the space in this chapter, asexual reproduction is responsible for many of the new plant individuals appearing on Earth. This fact suggests that in some circumstances, asexual reproduction must be advantageous.

Consider genetic recombination. When a plant self-fertilizes, there are fewer opportunities for genetic recombination than there are with cross-fertilization. A self-fertilizing plant that is heterozygous for a certain locus can produce among its progeny both kinds of homozygotes for that locus plus the heterozygote, but it cannot produce any progeny that carry alleles that it does not itself possess. Yet many plants continue to be self-compatible.

Asexual reproduction goes farther than self-fertilization: It eliminates genetic recombination altogether. When a plant reproduces asexually, it produces a clone of progeny with genotypes identical to its own. If a plant is well adapted to its

environment, asexual reproduction may spread its genotype throughout that environment. This ability to exploit a particular environment is an advantage of asexual reproduction.

There are many forms of asexual reproduction

We call stems, leaves, and roots vegetative organs, distinguishing them from flowers, the reproductive parts of the

plant. The modification of a vegetative organ is what makes vegetative reproduction possible. The stem is the organ that is modified in many cases. Strawberries and some grasses produce stolons (runners), horizontal stems that form roots at intervals and establish potentially independent plants (see Figure 34.4b). Tip layers are upright branches whose tips sag to the ground and put out roots, as in blackberry and forsythia.

Some plants, such as potatoes, form tubers, enlarged fleshy tips of underground stems (see Figure 34.4a). Rhizomes are horizontal underground stems that can give rise to new shoots. Bamboo is a striking example of a plant that reproduces vegetatively by means of rhizomes. A single bamboo plant can give rise to a stand—even a forest—of plants constituting a single, physically connected entity.

Whereas stolons and rhizomes are horizontal stems, bulbs and corms are short, vertical, underground stems. Lilies and onions form bulbs (Figure 38.17f), short stems with many fleshy, modified leaves. The leaves make up most of the bulb. Bulbs are thus large buds that store nutrients. They can give rise to new plants by dividing or by producing new bulbs from lateral buds. Crocuses, gladioli, and many other plants produce corms, underground stems that function very much as bulbs do. Corms are disklike and consist primarily of stem tissue; they lack the fleshy modified leaves that are characteristic of bulbs.

Not all vegetative organs modified for reproduction are stems. Leaves may also be the source of new plantlets, as in the succulent plants of the genus *Kalanchoe* (Figure 38.17b). Many kinds of angiosperms, ranging from grasses to trees such as aspens and poplars, form interconnected, genetically homogeneous populations by means of suckers — shoots produced by roots. What appears to be a whole stand of aspen trees, for example, may be a clone derived from a single tree by suckers (see Figure 54.1b).

Plants that reproduce vegetatively often grow in physically unstable environments, such as eroding hillsides. Plants with stolons or rhizomes, such as beach grasses,

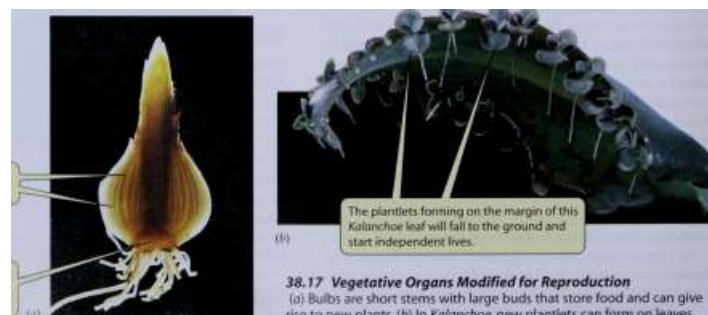
r \

Storage leaves grow

from the stem.

v

The short stem is visible at the bottom of the bulb.



38. 7 7 Vegetative Organs Modified for Reproduction

(a) Bulbs are short stems with large buds that store food and can give rise to new plants, (b) In *Kalanchoe*, new plantlets can form on leaves.

rushes, and sand verbena, are common pioneers on coastal sand dunes. Rapid vegetative reproduction enables these plants, once introduced, not only to multiply but also to survive burial by the shifting sand; in addition, the dunes are stabilized by the extensive network of rhizomes or stolons.

Dandelions, citrus trees, and some other plants reproduce by apomixis, the asexual production of seeds. As we have seen, meiosis reduces the number of chromosomes in gametes, and fertilization restores the sporophytic number of chromosomes in the zygote. Some plants can skip over both meiosis and fertilization and still produce seeds. Apomixis produces seeds within the female gametophyte without the mingling and segregation of chromosomes and without the union of gametes. The ovule simply develops into a seed, and the ovary wall develops into a fruit. An apomictic embryo has the sporophytic number (2n) of chromosomes. The result of apomixis is a fruit with seeds that are genetically identical to the parent plant.

Interestingly, apomixis sometimes requires pollination. In some apomictic species, a sperm nucleus must combine with the polar nuclei in order for the endosperm to form. In other apomictic species, the pollen provides the signals for embryo and endosperm formation, although neither sperm nucleus participates in fertilization. Pollination and fertilization are not the

same thing!

Asexual reproduction is important in agriculture

Farmers take advantage of some natural forms of vegetative reproduction. Farmers and scientists have also developed new types of asexual reproduction by manipulating plants. One of the oldest methods of vegetative reproduction used in agriculture consists simply of making cuttings of stems, inserting them in soil, and waiting for them to form roots and thus become autonomous plants. The cuttings are usually encouraged to root by treatment with a plant hormone, auxin, as described in Chapter 37.

Horticulturists reproduce many woody plants by grafting —attaching a bud or a piece of stem from one plant to the root-bearing stem of another plant. The part of the resulting plant that comes from the root-bearing "host" is called the stock; the part grafted on is called the scion (Figure 38.18). In order for a graft to succeed, the cambium of the scion must become associated with the cambium of the stock. By cell division, both cambia form masses of wound tissue. If the two masses meet and fuse, the resulting continuous cambium can produce xylem and phloem, allowing transport of water and minerals to the scion and of photosynthate to the stock. Grafts are most often successful when the stock and scion belong to the same or closely related species.

Most fruit grown for market in the United States is produced on trees grown from grafts. There are many reasons for grafting plants for fruit production. The most common is the desire to combine a hardy root system with a shoot system that produces the best-tasting fruit. This motive is

(a) Cleft grafting

Scions

(b) Whip grafting Scion



In grafting, the scions are aligned so that their vascular cambia associate with the vascular cambium in the stock.

38.18 Grafting

Grafting—attaching a piece of a plant to the stem or root of another plant—is common in agriculture. The "host" stem or root is the stock; the grafted piece is the scion.

illustrated by the story of the wine grape *Vitis vinifera*. In 1863, plant lice of the genus *Phylloxera* inflicted great damage on the root systems of grapevines in French vineyards. More than 2.5 million acres of vines were destroyed. The problem was overcome by importing *V. vinifera* plants, which have *Phylloxera*-resistant root systems, from California. These plants were used as stocks to which French vines were grafted as scions. Thus the fine French grapes could be grown using roots resistant to the lice. (But the battle continues; in recent years, a new strain of *Phylloxera* has been damaging grapevines in California.)

Scientists in universities and industrial laboratories have been developing new ways to produce valuable plant materials via tissue culture. Because many plant cells are totipotent (see Figure 16.3), cultures of undifferentiated tissue can give rise to entire plants, as can small pieces of tissue cut directly from a parent plant. Tissue cultures are used commercially to produce orchids, rhododendrons, and many crops without resorting to seeds.

Culturing tiny bits of apical meristem can produce plants free of viruses. Because apical meristems lack developed vascular tissues, viruses tend not to enter them. Such meristem cultures have been used to increase the yields of potatoes and other crops.

Recombinant DNA techniques applied to tissue cultures can provide plants with capabilities they previously lacked such as resistance to pests, or increased nutritive value to humans. There is also interest in making certain valuable, sexually reproducing plants capable of apomixis. By causing cells of different types to fuse, one can obtain plants with exciting new combinations of properties.

678 CHAPTER THIRTY-EIGHT

Chapter Summary

Many Ways to Reproduce

- ▶ Almost all flowering plants reproduce sexually, and many also reproduce asexually.
- ▶ Both sexual and asexual reproduction are important in agriculture.

Sexual Reproduction

- ▶ Sexual reproduction promotes genetic diversity in a population, which may give the population an advantage under changing environmental conditions.
- ▶ The flower is an angiosperm's device for sexual reproduction.
- ▶ Flowering plants have microscopic gametophytes that develop in the flowers of the sporophytes. The megagametophyte is the embryo sac, which typically contains eight nuclei in a total of seven cells. The microgametophyte is the pollen grain, which delivers two sperm cells to the megagametophyte by means of a long pollen tube. Review Figure 38.1
- ▶ Pollination enables fertilization in the absence of liquid water.
- ▶ In self-incompatible species, the stigma rejects pollen from the same plant. Review Figure 38.4
- ▶ Angiosperms perform double fertilization: One sperm nucleus fertilizes the egg, forming a zygote, and the other sperm nucleus unites with the two polar nuclei to form a triploid endosperm nucleus. Review Figure 38.6
- ▶ The zygote develops into an embryo (with an attached suspensor), which remains quiescent in the seed until conditions are right for germination. The endosperm is the nutritive reserve upon which the embryo depends at germination. Review Figures 38.7, 38.8
- ▶ Flowers develop into seed-containing fruits, which often play important roles in the dispersal of the species.

The Transition to the Flowering State

- ▶ For a vegetatively growing plant to flower, an apical meristem in the shoot system must become an inflorescence meristem, which gives rise to bracts and more meristems. The meristems it produces may become floral meristems or additional inflorescence meristems. Review Figure 38.10
- ▶ Flowering results from a cascade of gene expression. Organ identity genes are expressed in floral meristems that give rise to sepals, petals, stamens, and carpels.

Photoperiodic Control of Flowering

- ▶ Photoperiodic plants regulate their flowering by measuring the length of light and dark periods.
- ▶ Short-day plants flower when the days are shorter than a species-specific critical day length; long-day plants flower when the days are longer than a critical day length. Review Figure 38.11
- ▶ Some angiosperms have more complex photoperiodic requirements than short-day or long-day plants have, but most are day-neutral.
- ▶ The length of the night is what actually determines whether a photoperiodic plant will flower. Review Figure 38.12
- ▶ Interruption of the nightly dark period by a brief exposure to light undoes the effect of a long night. Review Figure 38.13
- ▶ The mechanism of photoperiodic control involves a biological clock and phytochromes. Review Figures 38.14, 38.15
- ▶ Evidence suggests that there is a flowering hormone, called florigen, but the substance has yet to be isolated from any plant. Review Figure 38.16

Vernalization and Flowering

- ▶ In some plant species, exposure to low temperatures—vernalization—is required for flowering.

-ver-

Asexual Reproduction

- ▶ Asexual reproduction allows rapid multiplication of organisms well suited to their environment.
- ▶ Vegetative reproduction involves the modification of a vegetative organ—usually the stem—for reproduction. Stolons, runners, tubers, rhizomes, bulbs, corms, and suckers are means by which plants may reproduce vegetatively.
- ▶ Some plant species produce seeds asexually by apomixis.
- ▶ Agriculturalists use natural and artificial techniques of asexual reproduction to reproduce particularly desirable plants.
- ▶ Horticulturists often graft different plants together to take advantage of favorable properties of both stock and scion. Review Figure 38.18
- ▶ Tissue culture techniques, based on the totipotency of many plant cells, are used to propagate plants asexually, to produce virus-free clones of crop plants, and to manipulate plants by recombinant DNA technology.

For Discussion

1. For a crop plant that reproduces both sexually and asexually, which method of reproduction might the farmer prefer?
2. Thompson seedless grapes are produced by vines that are triploid. Think about the consequences of this chromosomal condition for meiosis in the flowers. Why are these grapes seedless? Describe the role played by the flower in fruit formation when no seeds are being formed. How do you suppose Thompson seedless grapes are propagated?
3. Poinsettias are popular ornamental plants that typically bloom just before Christmas. Their flowering is photoperiodically controlled. Are they long-day or short-day plants? Explain.
4. You plan to induce the flowering of a crop of long-day plants in the field by using artificial light. Is it necessary to keep the lights on continuously from sundown until the point at which the critical day length is reached?

39

Plant Responses to Environmental Challenges

^pr?v" " If you are attacked, it makes sense to

M call for help. Plants do this, too. When caterpillars

WF begin to chew on the leaves of corn, cotton, or some other plant species, the plants synthesize and release chemical signals into the atmosphere. These substances attract other insects that feed on the caterpillars.

Herbivores aren't the only challenges plants face, however. The environment teems with plant pathogens. We know of more than a hundred diseases that can kill a tomato plant, each of them caused by a different pathogen (including various bacteria, fungi, protists, and viruses). Like animals, plants have a variety of defenses against pathogens. And, like the defenses of our own bodies, these mechanisms are not perfect, but they keep the plant world in competitive balance with its pathogens.

Environmental challenges to plants aren't limited to herbivores and pathogens. Some physical conditions pose substantial problems for plants and thus limit the places where different kinds of plants can live. The most challenging physical environments include ones that are very dry (deserts), that are water-saturated, that are dangerously salty, that contain high concentrations of toxic substances such as heavy metals, and that are very hot or very cold.

This chapter focuses on how plants meet the myriad challenges presented by their biological and physical environments. We begin by examining interactions between plants and pathogens and go on to consider interactions between plants and herbivores. Then we discuss the adaptations of some types of plants to their physical environments.

Plant-Pathogen Interactions

Plants and pathogens have evolved together in a continuing "arms race." Pathogens have evolved mechanisms by which to attack plants, and plants have evolved defenses against them. Each set of mechanisms uses information from the other. The pathogen's enzymes break down the plant's cell walls, for example, and the breakdown products signal to the plant that it is under attack. In turn, the plant's defenses alert the pathogen that it is under attack.

Calling In an Air Strike

As this caterpillar of a corn earworm moth (*Helicoverpa zed*) munches on a cotton boll, it is triggering a series of reactions in the plant that may end in the attraction of other insects that will attack the caterpillar.

What determines the outcome of a battle between a plant and a pathogen? The key to success for the plant is to respond to the information about the pathogen quickly and massively. Plants use both mechanical and chemical defenses in this effort.

Plants seal off infected parts to limit damage

Tissues such as epidermis or cork protect the outer surfaces of plants, and these tissues are generally covered by cutin, suberin, or waxes. This protection is comparable to the nonspecific defenses of animals. When pathogens pass these barriers, other nonspecific plant defenses are activated.

The defense systems of plants and animals differ. Animals generally repair tissues that have been damaged by pathogens, but plants do not. Instead, they seal off and sacrifice the damaged tissue so that the rest of the plant does not become infected. This approach works because most plants, unlike most animals, are modular and can replace damaged parts by growing new ones.

One of a plant cell's first defensive responses is the rapid deposition of additional polysaccharides to the cell wall, reinforcing this barrier to invasion by the pathogen (Figure 39.1). These polysaccharides block the plasmodesmata, limiting the ability of viral pathogens to move from cell to cell. They also serve as a base upon which lignin may be laid down. Lignin enhances the mechanical barrier, and the toxicity of lignin building blocks makes the cell inhospitable to some pathogens.



680 CHAPTER THIRTY-NINE

Pathogen molecules

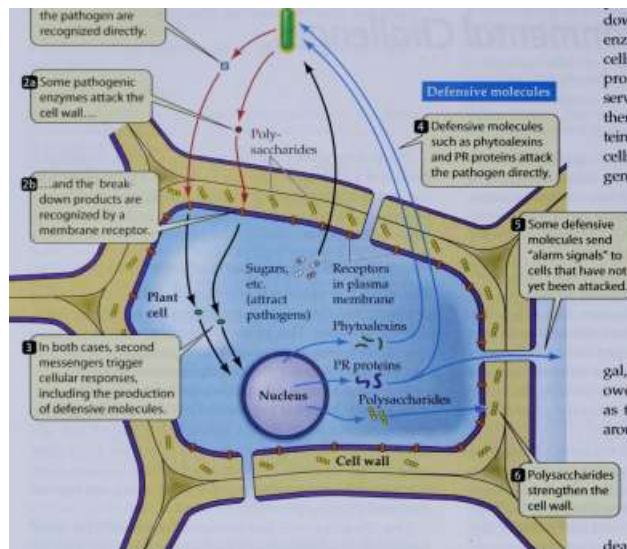
Q Some molecules from the pathogen are recognized directly.

Pathogen

^Some pathogenic enzymes attack the cell wall...

Defensive molecules

.and the breakdown products are recognized by a membrane receptor.



Plants also produce several types of pathogenesis-related proteins, or PR proteins. Some are enzymes that break down the cell walls of pathogens. These enzymes destroy some of the invading cells, and in some cases the breakdown products of the pathogen's cell walls serve as chemical signals that trigger further defensive responses. Other PR proteins may serve as alarm signals to plant cells that have not yet been attacked. In general, PR proteins appear not to be rapid-response weapons; rather, they act more slowly, perhaps after other mechanisms have blunted the pathogen's attack.

I Polysaccharides strengthen the cell wall.



39. 7 Signaling between Plants and Pathogens

Chemical interactions between plants and pathogens are highly coevolved. Plants produce molecules such as sugars that attract pathogens. But the presence of a pathogen stimulates the plant to produce defensive molecules that can work in many different ways.

Plants have potent chemical defenses against pathogens

When infected by certain fungi and bacteria, plants produce a variety of defensive compounds, among which are small molecules called phytoalexins and larger proteins called pathogenesis-related proteins (see Figure 39.1).

Phytoalexins are toxic to many fungi and bacteria. (Most are phenolics or terpenes, compounds that are also used to protect plants against herbivores; see Table 39.1.) They are produced by infected cells and their immediate neighbors within hours of the onset of infection. Enzymes from a pathogenic fungus can cause plant cell walls to release hormones called oligosaccharins (see Chapter 37), which trigger phytoalexin production. Because their antimicrobial activity is nonspecific, phytoalexins can

destroy many species of fungi and bacteria in addition to the one that originally triggered their production. Physical injuries, viral infections, and chemical compounds produced in response to damage by herbivores can also induce the production of phytoalexins.

The hypersensitive response is a localized containment strategy

Plants that are resistant to fungal, bacterial, or viral diseases generally owe this resistance to what is known as the hypersensitive response. Cells around the site of microbial infection die, preventing the spread of the pathogen by depriving it of nutrients. Some of the cells produce phytoalexins and other chemicals before they die. The dead tissue, called a necrotic lesion, contains and isolates what is left of the microbial invasion (Figure 39.2). The rest of the plant remains free of the infecting microbe.

One of the chemicals produced during the hypersensitive response is a close relative of aspirin. Since ancient times, people in Asia, Europe, and the Americas have used willow (*Salix*) leaves and bark to relieve pain and fever. The active ingredient in willow is salicylic acid, the same substance from which aspirin is derived:

Salicylic acid

OH

It now appears that all plants contain at least some salicylic acid. This compound plays a hormonal role in the plants' own defenses, often leading to a long-lasting effect that makes them resistant to later attacks by pathogens.

Systemic acquired resistance is a form of long-term "immunity"

Systemic acquired resistance is a general increase in the resistance of the entire plant to a wide range of pathogen species. It is not limited to the pathogen that originally triggered it or to the site of the original infection.



39.2 The Aftermath of a Hypersensitive Response

The necrotic spots on these leaves are a response to the fungus that causes strawberry blight.

The systemic acquired resistance that sometimes follows the hypersensitive response is accompanied by the synthesis of PR proteins. Treatment of plants with salicylic acid or aspirin leads to the production of PR proteins and to a resistance to pathogens. Salicylic acid treatment provides substantial protection against tobacco mosaic virus (a well-studied plant pathogen) and some other viruses.

Salicylic acid also serves as a hormone for disease resistance. In some cases, microbial infection in one part of a plant leads to the export of salicylic acid to other parts of the plant, where it causes the production of PR proteins before the infection can spread. The PR proteins then limit the extent of the infection. Infected plant parts also produce the closely related methyl salicylate (also known as oil of winter-green). This volatile substance travels to other plant parts through the air. It may be that methyl salicylate can also trigger the production of PR proteins in neighboring plants that have not yet been infected.

Some plant genes match up with pathogen genes

Many plants use the hypersensitive response and systemic acquired resistance as nonspecific defenses against various pathogens. However, the triggering of these responses resides in a highly specific mechanism, called gene-for-gene resistance. In gene-for-gene resistance, the ability of a plant to defend itself against a specific strain of

PLANT RESPONSES TO ENVIRONMENTAL CHALLENGES 681

a pathogen depends on the plant's having a particular allele of a gene that corresponds to a particular allele of a gene in the pathogen (Figure 39.3). Let's see how this matching works.

Plants have a large number of R genes (resistance genes), and many pathogens have sets of Avr genes (avirulence genes). Dominant R alleles favor resistance, and dominant Avr alleles make a pathogen less effective. If a particular plant has the dominant allele of an R gene and a pathogen strain infecting it has the dominant allele of the corresponding Avr gene, the plant will be resistant to that strain. This is true even when none of the other R-Avr pairs features corresponding dominant alleles. (This effect, one R-Avr pair overruling the others, is an example of epistasis, which was discussed in Chapter 10.)

The mechanism of gene-for-gene resistance is not completely understood. There are thousands of specific R genes among the plants, and their products have different functions. The Avr genes in pathogens are simply genes that cause the pathogen to produce a substance that elicits a defensive response in the plant. Most gene-for-gene interactions trigger the hypersensitive response.

Not all biological threats to plants come from microorganisms and viruses that cause diseases. Many animals, from

inchworms to elephants, eat plants.

Plants and Herbivores: Benefits and Losses

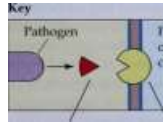
Herbivores—animals that eat plants—depend on plants for energy and nutrients. Plants have many defense mechanisms that protect them against herbivores, as we will see. First, let's consider how herbivores can have a positive effect on the plants they eat.

Grazing increases the productivity of some plants

In grazing, a predator eats part of a plant, such as the leaves, without killing its prey, which then has the potential

to grow back (Figure 39.4). What are the consequences of grazing? Is it always detrimental to plants, or are they somehow

Plant host cell resistance gene



Plant

cell

cytoplasm

Dominant

allele

present

IT

Pathogen

avirulence

gene

Dominant

allele

present

Signal encoded by dominant allele of pathogen.

Receptor, encoded by dominant allele, in plant cell plasma membrane.

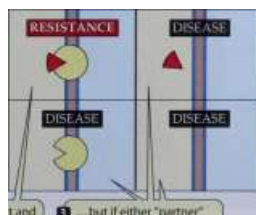
Dominant allele absent

Dominant allele absent

39.3 Gene-for-Gene Resistance

A single pair of corresponding dominant alleles promotes resistance even if all the other pairs are mismatches.

If, in any pair, the host and pathogen both contain a dominant allele, the plant will resist the pathogen...



... but if either "partner" lacks the dominant allele, this pair will not cause gene-for-gene resistance.

adapted to their place in the food chain? Certain plants and their predators evolved together, each acting as the agent of natural selection on the other (see Chapter 29). Because of this coevolution, grazing actually increases photo-synthetic production in some plant species.

Removing some leaves from a plant may increase the rate of photosynthesis of the remaining leaves. This phenomenon probably is the result of several factors. First, nitrogen obtained from the soil by the roots no longer needs to be divided among so many leaves. Second, the export of sugars and other photosynthetic products from the leaves may be enhanced because the demand for those products in the roots is undiminished, while the sources for such products—leaves—have been decreased. The remaining leaves may compensate by photosynthesizing more rapidly.

A third and particularly significant factor increasing photosynthesis, especially in grasses, is an increase in the availability of light to the younger, more active leaves or leaf parts. The removal of older or dead leaves by a grazer decreases the shading of younger leaves. Unlike most other plants, which grow from their shoot and leaf tips, grasses grow from the base of the shoot and leaf, so their growth is not cut short by grazing.

Mule deer and elk graze many plants, including one called scarlet gilia. Their grazing removes about 95 percent of the aboveground part of each plant (Figure 39.5), but each plant quickly regrows not one, but four, replacement stems. Grazed plants produce three times as many fruits by the end of the growing season as do ungrazed plants.



EXPERIMENT

Question: Is grazing by herbivores always detrimental to a plant?

A scarlet gilia was cropped to the point indicated.

The cropped plant grew four new stems and produced almost three times as many offspring...

...as did uncropped control plants.



Conclusion: Cropping can lead to increased growth.

39.4 Is Grazing Helpful or Harmful to Plants?

Grazing mammals such as this North American elk exist in virtually all of Earth's biomes, and the plants they feed on have evolved along with them.

39.5 Overcompensation for Being Eaten

Experiments confirm that some plants benefit from the effects of grazing.

Some grazed trees and shrubs continue to grow until much later in the season than do ungrazed but otherwise similar plants. This longer growing season results in part because the removal of apical buds by the grazers stimulates lateral buds to become active, producing a more heavily branched plant. Leaves on ungrazed plants may also die earlier in the growing season than leaves on grazed plants.

A plant may benefit from moderate herbivory by attracting animals that spread its pollen or that eat its fruit and thus

disperse its seeds. Nevertheless, resisting attack by herbivores is often to the advantage of a plant.

Some plants produce chemical defenses

Although a plant cannot flee its herbivorous enemies, it may be able to defend itself chemically. Many plants attract, resist, and inhibit other organisms by producing special chemicals known as secondary products. Primary products are substances, such as proteins, nucleic acids, carbohydrates, and lipids, that are produced and used by all living things. Although all organisms use the same kinds of primary products, plants can differ as radically in their secondary products as they do in their external appearance.

The more than 10,000 known secondary plant products range in molecular weight from about 70 to more than 400,000, but most are of low molecular weight. Some are produced by only a single species, while others are characteristic of an entire genus or even family. These compounds help plants compensate for being unable to move.

PLANT RESPONSES TO ENVIRONMENTAL CHALLENGES 683

Jy, I Secondary Plant Products in Defense

CLASS

TYPE

ROLE

EXAMPLE

Nitrogen-containing

Phenolics

Terpenes

Alkaloids

Glycosides

Nonprotein amino acids

Flavonoids

Quinones

Tannins

Monoterpenes

Sesquiterpenes Steroids

Polyterpenes

Affect herbivore nervous system

Release cyanide or sulfur compounds

Disrupt herbivore protein structure

Phytoalexins

Inhibit competing plants

Herbivore and microbe deterrents

Insecticides

Antiherbivores

Mimic insect hormones and disrupt

insect life cycle Feeding deterrent?

Nicotine in tobacco Dhurrin in sorghum Canavanine in jack bean Capsidol in peppers Juglone in walnut Many woods, such as oak Pyrethroids in

chrysanthemum Gossypol in cotton

Rubber in rubber tree

The effects of defensive secondary products on animals are diverse. Some secondary products act on the nervous systems of herbivorous insects, mollusks, or mammals. Others mimic the natural hormones of insects, causing some larvae to fail to develop into adults. Still others damage the digestive tracts of herbivores. Some secondary products are toxic to fungal pests. Humans make commercial use of many secondary plant products as fungicides, insecticides, rodenticides, and pharmaceuticals.

While many secondary products have protective functions, others are essential as attractants for pollinators and seed dispersers. Table 39.1 lists the major classes of defensive secondary plant products and their biological roles.

Let's look at a specific example of an insecticidal secondary product, canavanine.

Some secondary products play multiple roles

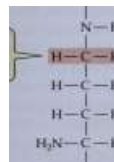
Canavanine is an amino acid that is not found in proteins, but is closely similar to the amino acid arginine, which is found in almost all proteins. Canavanine has two important roles in those plants that produce it in significant quantities. The first role is as a nitrogen-storing compound in seeds. The second, defensive role is based on the similarity of canavanine to arginine:

NH-

C = NH

NH

A seemingly slight chemical difference...



...produces

inactive

proteins.

C—OH

O

Arginine

H₂N—C

C—OH

H₂O

Canavanine

Many insect larvae that consume canavanine-containing plant tissue are poisoned. The canavanine is incorporated into the insect's proteins in some of the places where the DNA has coded for arginine because the enzyme that charges the tRNA specific for arginine fails to discriminate accurately between the two amino acids. The structure of canavanine is different enough from that of arginine that some of the resulting proteins end up with a modified tertiary structure and hence reduced biological activity. These defects in protein structure and function lead to developmental abnormalities that kill the insect.

A few insect larvae are able to eat canavanine-containing plant tissue and still develop normally. How can this be? In these larvae, the enzyme that charges the arginine tRNA discriminates correctly between arginine and canavanine. The canavanine they ingest is thus not incorporated into the proteins they form, and the larvae are not harmed.

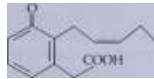
Many defenses depend on extensive signaling

Plant defenses result from a series of signals. Insects feeding on tomato leaves damage the cells, leading to a chain of events including the formation of hormones and ending with the production of an insecticide. The signaling steps in the production of one defensive compound, shown in Figure 39.6, involve two hormones. Systemin is a polypeptide hormone—the first polypeptide hormone to be discovered in plants. Jasmonates are formed from the unsaturated fatty acid linolenic acid. The

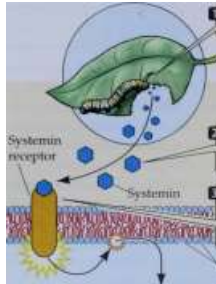
final step in this series is the production of a protease inhibitor. The inhibitor, once in an insect's gut, interferes with the digestion of proteins and thus stunts the insect's growth.

Jasmonic acid (a jasmonate)

Jasmonates also take part in the "call for help" described at the beginning of this chapter. In that case, a substance re-



684 CHAPTER THIRTY-NINE



A herbivore wounds the leaf...

Outside of cell

Systemin receptor

V J 7wf

...causing the production of systemin, a polypeptide hormone.

Systemin travels to other parts of the plant and binds a receptor in the plasma membrane...

Membrane phospholipids

Cytoplasm

Product of

lipid breakdown , ^_E1

\

Jasmonates <^X^>

"A



.causing a membrane phospholipid to break down, forming a product that is converted to jasmonates, which are also hormones.

V

T

Gene activation/

\ A

Protease inhibitor

Q Jasmonates enter the nucleus and activate genes to form the protease inhibitor that poisons herbivores.

Nucleus

39.6 A Signaling Pathway for Synthesis of an Insecticide

The chain of events initiated by an insect's attack and leading to the production of a defensive chemical can consist of many steps. These steps may include the synthesis of one or two hormones, binding of receptors, gene activation, and, finally, synthesis of a poison.

leased by chewing caterpillars is the first signal, leading to the formation of jasmonates by the plant. The jasmonates, in turn, trigger the formation of the volatile compounds that attract the insects that prey on the caterpillars.

Gene splicing may confer resistance to insects

Wild and domesticated common beans (*Phaseolus vulgaris*) differ in their resistance to attack by two species of bean weevils. Some wild bean seeds are highly resistant to these insects, but no cultivated bean seeds show such resistance. Scientists discovered that all weevil-resistant bean seeds contain a specific seed protein, arcelin. This protein has never been found in cultivated bean seeds. Therefore, the scientists hypothesized that arcelin is responsible for the resistance of some seeds to predation by the weevils.

To rule out other differences between wild and cultivated beans as being responsible for the resistance, the scientists performed two series of experiments. In one series, they crossed cultivated and wild bean plants. All of the progeny seeds of such crosses that contained arcelin showed resistance to weevils. In the other series of experi-

ments, the scientists worked with artificial bean seeds made by removing the seed coats of cultivated beans and grinding the remainder of the seeds into flour. They added different concentrations of arcelin to different batches and molded the flour into artificial seeds. They then let weevils attack the artificial seeds. The more arcelin the artificial seeds contained, the more resistant they were to weevils.

In preliminary tests, arcelin in cooked beans was shown not to be harmful to rats—a first step toward determining whether arcelin is safe in food for humans. Agricultural scientists must sometimes choose between crop protection and appeal to humans. A plant with sturdy chemical defenses may taste bad, make us sick, or even kill us.

The development of crop plants that produce their own pesticides is an active area of research in agricultural biotechnology. Scientists are seeking to introduce genes for arcelin and other resistance-conferring proteins into agriculturally important crops such as beans. One of the most widely applied approaches is the engineering of several crops, such as tomato, corn, and cotton, to express the toxin genes from *Bacillus thuringiensis* discussed in Chapter 17.

Why don't plants poison themselves?

Why don't the chemicals that are so toxic to herbivores and microbes kill the plants that produce them? Plants that produce toxic secondary products generally use one of the following measures to protect themselves:

- ▶ The toxic material is isolated in a special compartment, such as the central vacuole.
- ▶ The toxic substance is produced only after the plant's cells have already been damaged.
- ▶ The plant uses modified enzymes or modified receptors that do not recognize the toxic substance.

The first method is the most common. Plants using this method store their poisons in vacuoles if they are water-soluble. If hydrophobic, the poisons are stored in laticifers (tubes containing a white, rubbery latex) or dissolved in waxes on the epidermal surface. This compartmentalized storage keeps the toxic substance away from the mitochondria, chloroplasts, and other parts of the plant's own metabolic machinery.

Some plants store the precursors of toxic substances in one compartment, such as the epidermis, and store the enzymes that convert the precursors to the active poison in another compartment, such as the mesophyll. These plants produce the toxic substance only after being damaged. When an herbivore chews part of the plant, the cells rupture, and the enzymes come in contact with the precursors, producing the toxic product. The only part of the plant that is damaged by the toxic material is that which was already damaged by the herbivore. Plants that respond to attack by producing cyanide—a strong inhibitor of cellular respiration in all organisms that respire—are among those that use this protective measure.

The third protective measure is used by the canavanine-producing plants described earlier. These plants produce a



39.7 Disarming a Plant's Defenses

This beetle is inactivating a milkweed's defense system by cutting its laticifer supply lines.

tRNA-charging enzyme for arginine that does not bind canavanine. However, as we have seen, some herbivores can evade being poisoned by canavanine in a similar manner, demonstrating that no plant defense is perfect.

The plant doesn't always win

Milkweeds such as *Asclepias syriaca* are latex-producing (laticiferous) plants. When damaged, a milkweed releases copious amounts of toxic latex from its laticifers. Latex has long been suspected to deter insects from eating the plant, because insects that feed on neighboring plants of other species do not attack laticiferous plants. This observed behavior is consistent with, but does not prove, the hypothesis that the latex keeps the insects at bay.

Stronger support for the hypothesis was obtained by studying field populations of *Labidomera clivicollis*, a beetle that is one of the few insects that feed on *A. syriaca*. These beetles show a remarkable prefeeding behavior: They cut a few veins in the leaves before settling down to dine (Figure 39.7). Cutting the veins, with their adjacent laticifers, causes massive latex leakage and interrupts the latex supply to a downstream portion of the leaf. The beetles then move to the relatively latex-free portion and eat their fill.

Does this behavior of the beetles negate the adaptive value of latex protection? Not entirely. There are still great numbers of potential insect pests that are effectively deterred by the latex. And evolution proceeds. Over time, milkweed plants producing higher concentrations of toxins may be selected by virtue of their ability to kill beetles that cut their laticifers.

Having discussed how plants defend themselves against other organisms, we now turn our attention to how plants adapt to environments where water is a problem.

Water Extremes: Dry Soils and Saturated Soils

Water is often in short supply in the terrestrial environment. Some terrestrial habitats, such as deserts, intensify

this challenge, and many plants that inhabit particularly dry areas have one or more adaptations that allow them to conserve water. Plants adapted to dry environments are called xerophytes.

Some plants evade drought

Some desert plants have no special structural adaptations for water conservation other than those found in almost all flowering plants. Instead, they have an alternative strategy. These desert annuals simply evade the periods of drought. They carry out their entire life cycle—from seed to seed—during a brief period in which rainfall has made the surrounding desert soil sufficiently moist (Figure 39.8).

Some leaves have special adaptations to dry environments

Plants that remain active during dry periods must have structural adaptations that enable them to survive. The secretion of a heavier cuticle over the leaf epidermis to retard water loss is a common adaptation to dry environments. An even more common adaptation is a dense covering of epidermal hairs. Some species have stomata only in sunken



39.8 Desert Annuals Evade Drought

Seeds of desert plants often lie dormant for long periods awaiting conditions appropriate for germination. When they do germinate, they grow and reproduce rapidly before the short wet season passes. During the long dry spells, only seeds remain alive.



39.9 Stomatal Crypts

Stomata in the leaves of some xerophytes are in sunken pits called stomatal crypts. The hairs covering these crypts trap moist air. (A section of the leaf's interior can be seen at the top of the photo.)

During dry periods, the thorny, leafless stems of an ocotillo appear almost dead.



When water is on hand, leaves develop rapidly and provide the plant with photosynthetic products.

39.10 Opportune Leaf Production

The ocotillo, a xerophyte that lives in the lower deserts of the southwestern United States and northern Mexico, produces leaves only when there is sufficient water for photosynthesis.

cavities below the leaf surface, which reduces the drying effects of air currents; often these stomatal cavities contain hairs as well (Figure 39.9).

Succulence—the possession of fleshy, water-storing leaves—is an adaptation to dry environments. Ice plants and their relatives have fleshy leaves in which water may be stored. Others, such as ocotillo, produce leaves only when water is abundant, shedding them as the soil dries out (Figure 39.10). Cacti and similar plants have spines rather than typical leaves, and photosynthesis is confined to the fleshy stems. The spines may reflect incident radiation, or they may dissipate heat. Corn and some related grasses have leaves that roll up during dry periods, thus reducing the leaf surface area through which water is lost. Some trees, such as eucalyptuses, that grow in arid regions have leaves that hang vertically at all times, thus evading the midday sun (Figure 39.11).

Xerophytic adaptations of leaves minimize water loss by the plant. However, such adaptations simultaneously minimize the uptake of carbon dioxide and thus limit photosynthesis. In consequence, most xerophytes grow slowly, but they utilize water more efficiently than do other plants; that is, they fix more grams of carbon by photosynthesis per gram of water lost to transpiration than other plants do.

Plants have other adaptations to a limited water supply

Roots may also be adapted to dry environments. The Atacama Desert in northern Chile often goes several years without measurable rainfall. The landscape there is almost barren of plant life, save for many surprisingly large



39.7 7 Shade at Midday

Because eucalyptus leaves hang vertically, their flat surfaces are not presented directly to the midday sun. This adaptation minimizes heating as well as water loss.

PLANT RESPONSES TO ENVIRONMENTAL CHALLENGES 687

mesquite trees (genus *Prosopis*; Figure 39.12). These trees obtain water through taproots that grow to great depths, reaching water supplies far underground, as well as from condensation on their leaves.

A more common adaptation of desert plants is a root system that grows rapidly during rainy seasons but dies back during dry periods. Cacti have shallow but extensive fibrous root systems that effectively intercept water at the surface of the soil following even light rains.

Xerophytes and other plants that receive inadequate water may accumulate the amino acid proline to substantial concentrations in their vacuoles. As a consequence, the osmotic potential and water potential of their cells become more negative; thus these plants tend to extract more water from the soil than do plants that lack this adaptation. Plants living in salty environments share this and several other adaptations with xerophytes, as we will see.

As we have seen, there are many ways in which some plants eke out an existence in terrestrial environments with very little water. What happens if there is too much water?

In water-saturated soils, oxygen is scarce

Some plants live in environments so wet that the diffusion of oxygen to their roots is severely limited. Since most plant roots require oxygen to support respiration and ATP production, most plants cannot tolerate this situation for long.



Pneumatophores are root extensions that grow out of the water, under which the rest of the roots are submerged.

39.13 Coming Up for Air

The roots of the mangroves in this tidal swamp obtain oxygen through pneumatophores.



39.12 Mining Water with Deep Taproots

Death Valley, California, is not as arid as the Chilean Atacama, but the mesquite must reach far down into the sand dunes for its water supply.

Some species, however, are adapted to life in a water-saturated habitat. Their roots grow slowly and hence do not penetrate deeply. Because the oxygen level is too low to support aerobic respiration, the roots carry on alcoholic fermentation (see Chapter 7), which provides ATP for the activities of the root system but explains why growth is slow.

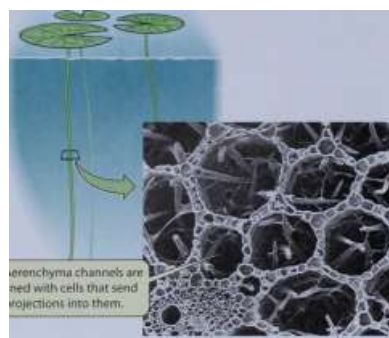
The root systems of some plants adapted to swampy environments have pneumatophores, which are extensions that grow out of the water and up into the air (Figure 39.13). Pneumatophores have lenticels and contain spongy tissues that allow oxygen to diffuse through them, aerating the submerged parts of the root system. Cypresses and some mangroves are examples of plants with pneumatophores.

Submerged or partly submerged aquatic plants often have large air spaces in the leaf parenchyma and in the petioles. Tissue containing such air spaces is called aerenchyma (Figure 39.14). Aerenchyma stores oxygen produced by photosynthesis and permits its ready diffusion to parts of the plant where it is needed for cellular respiration.

Aerenchyma also imparts buoyancy. Furthermore, because it contains far fewer cells than most other plant tissue, respiratory metabolism in aerenchyma proceeds at a lower rate, and the need for oxygen is much reduced.

Thus far we have considered water supply—either too little or too much—as a factor limiting plant growth. Other substances also can make an environment inhospitable to plant growth. One of these is salt.

688 CHAPTER THIRTY-NINE



Aerenchyma channels are lined with cells that send projections into them.

Vascular bundle

Open channel

39.14 Aerenchyma Lets Oxygen Reach Submerged Tissues

The scanning electron micrograph, a cross section of a petiole of the yellow water lily, shows a vascular bundle and aerenchyma.

Too Much Salt: Saline Environments

Worldwide, no toxic substance restricts flowering plant growth more than salt (sodium chloride) does. Saline — salty — habitats support, at best, sparse vegetation. Saline habitats themselves are diverse, ranging from hot, dry, salty deserts to moist, cool, salty marshes. Along the seashore are saline environments created by ocean spray. The ocean itself is a saline environment, as are river estuaries, where fresh and salt water meet and mingle. The salinization of agricultural land is an increasing global problem. Even where crops are irrigated with fresh water, sodium ions from the water accumulate in the

soil to ever greater concentrations as the water evaporates.

Saline environments pose an osmotic problem for plants. Because of its high salt concentration, a saline environment has an unusually negative water potential. To obtain water from such an environment, a plant must have an even more negative water potential than that of a plant in a nonsaline environment; otherwise, it will lose water, wilt, and die. A second problem is the potential toxicity of high concentrations of certain ions, notably sodium and chloride.

The halophytes—plants adapted to saline habitats—belong to a wide variety of flowering plant groups. How can these plants cope with a highly saline environment?

Most halophytes accumulate salt

Most halophytes share one adaptation: They accumulate sodium and, usually, chloride ions, and transport these ions to the leaves. The accumulated ions are stored in the central vacuoles of leaf cells, away from more sensitive parts of the cells. Nonhalophytes accumulate relatively little sodium, even when placed in a saline environment; of the sodium

that is absorbed by their roots, very little is transported to the shoot. The increased salt concentration in the tissues of halophytes makes their water potential more negative, so they can take up water more easily from the saline environment.

In 1999, scientists reported the first success in causing overexpression of a gene in *Arabidopsis* that enables sodium uptake. This gene encodes a Na^+/H^+ antiport protein in the tonoplast (the membrane surrounding the central vacuole). By making the gene produce a greater than normal number of these antiport proteins, the scientists increased sodium transport in *Arabidopsis*, converting this nonhalophyte into a halophyte. Further research along this line may result in a great boost to agriculture in saline environments. Biologists in Israel and elsewhere have had some success in breeding crops that can be watered with seawater or diluted seawater.

Some halophytes have other adaptations to life in saline environments. Some, for example, have salt glands in their leaves. These glands excrete salt, which collects on the leaf surface until it is removed by rain or wind (Figure 39.15). This adaptation, which reduces the danger of poisoning by accumulated salt, is found both in some desert plants, such as tamarisk, and in some mangroves growing in seawater in the Tropics.

Salt glands can play multiple roles, as in the desert shrub *Atriplex halimus*. This shrub has glands that secrete salt into small bladders on the leaves, where, by increasing the gradient in water potential, the salt helps the leaves obtain water from the roots. At the same time, by making the water potential of the leaves more negative, the salt reduces the transpirational loss of water to the atmosphere.

The adaptations we have just discussed are specific to halophytes. Several other adaptations are shared by halophytes and xerophytes.

Halophytes and xerophytes have some similar adaptations

Many halophytes, like some xerophytes, accumulate the amino acid proline in their cell vacuoles, making the water



39.75 Secreting Salt

This salty mangrove has special salt glands that secrete salt, which appears here as crystals on the leaves.

potential of their tissues more negative. Unlike sodium, proline is relatively nontoxic.

Succulence is another adaptation that halophytes and xerophytes have in common, as might be expected, since saline environments, like dry ones, make water uptake difficult. Succulence characterizes many halophytes that occupy salt marshes. There the salt concentration in the soil solution may change throughout the day; while the tide is out, for instance, evaporation increases the salt concentration. Succulence may offer a reserve of water for the plant during the period of maximum salinity; when the salinity drops as the tide comes in, the leaf's store of water is replenished. Many succulents—both xerophytes and halophytes—use crassulacean acid metabolism (CAM) and have reversed stomatal cycles that enable them to conserve water by closing their stomata in the daytime (see Figure 35.11). Other general adaptations to a saline environment include high root-to-shoot ratios, sunken stomata, reduced leaf areas, and thick cuticles.

Salt is not the only toxic solute found in soils. Some heavy metal ions are more toxic than sodium at equivalent concentrations.

Habitats Laden with Heavy Metals

High concentrations of some heavy metal ions, such as aluminum, mercury, lead, and cadmium, poison most plants. Some geographic sites are naturally rich in heavy metals as a result of normal geological processes. Acid rain leads to the release of

toxic aluminum ions in the soil. Other human activities, notably the mining of metallic ores, leave localized areas—known as tailings—with substantial concentrations of heavy metals and low concentrations of nutrients. Such sites are hostile to most plants, and seeds falling on them generally do not produce adult plants.

Mine tailings rich in heavy metals, however, generally are not completely barren (Figure 39.16). They may support healthy plant populations that differ genetically from populations of the same species on the surrounding normal soils. How can these plants survive?

Initially, some plants were thought to tolerate heavy metals by excluding them: By not taking up the metal ions, it was believed, the plant avoided being poisoned. However, measurements have shown that tolerant plants growing on mine tailings do take up heavy metals, accumulating them to concentrations that would kill most plants. Thus the tolerant plants must have a mechanism for dealing with the heavy metals they take up. Such tolerant plants may be found to be useful agents for bioremediation, a decontamination process by which the heavy metal content of some contaminated soils is decreased by living organisms.

We know the mechanism of at least one case of tolerance to a heavy metal. The roots of a buckwheat grown in China secrete oxalic acid soon after they are exposed to aluminum concentrations high enough to inhibit root growth in other plants. Oxalic acid combines with aluminum ions, forming a complex that does not inhibit growth.

'si*&ttpt^^^&^^z%?^^^*^^^<^^



39.16 Life after Strip Mining

Although high concentrations of heavy metals kill most plants, grass is colonizing this eroded strip mine in North Park, Colorado.

From mine to mine, the heavy metals in the soil differ. In Wales and Scotland, bent grass (*Agrostis*) grows near many mines. Samples of bent grass from several such sites were tested for their ability to grow in various solutions, each containing only one heavy metal. In general, the plants tolerated a particular heavy metal—the one most abundant in their habitat—but were sensitive to others. That is, they tolerated only one or two heavy metals, rather than heavy metals as a group.

Tolerant plant populations can evolve and colonize an area surprisingly rapidly. The bent grass population around a particular copper mine in Wales is resistant to copper and is relatively abundant, even though the copper-rich soil dates from mining done only a century ago.

Hot and Cold Environments

Temperatures that are too high or too low can stress plants and even kill them. Plants differ in their sensitivity to heat and cold, but all plants have their limits.

Any temperature extreme can damage cellular membranes:

- High temperatures destabilize membranes and denature many proteins, especially some of the enzymes of photosynthesis.
- Low temperatures cause membranes to lose their fluidity and alter their permeabilities to solutes.
- Freezing temperatures may cause ice crystals to form, damaging cellular membranes.

690 CHAPTER THIRTY-NINE

Plants have ways of coping with high temperatures

Transpiration, the evaporative loss of water, can cool a plant, but it also increases the plant's need for water. Therefore, it is not surprising that many plants living in hot environments have adaptations similar to those of xerophytes. These adaptations include epidermal hairs and spines that radiate heat, modified leaf displays that intercept less direct sunlight, and others.

Plants respond within minutes to high temperatures by producing several kinds of heat shock proteins. Among these are

chaperonins (see Chapter 3), which help other proteins maintain their structures and avoid denaturation. Threshold temperatures for the production of heat shock proteins vary, but 40°C is sufficient to induce them in most plants. We have much to learn about the dozens of heat shock proteins, but we do know that some other types of stress also induce their formation. Among these are chilling and freezing.

Some plants are adapted to survival at low temperatures

Low temperatures above freezing injure many plants, including important crops such as rice, corn, and cotton. Many plant species can be modified to resist the effects of cold spells by a process called cold-hardening, which involves repeated exposure to cool, but not injurious, temperatures. The hardening process is a slow one, requiring many days. A key change that occurs during the hardening process is an increase in the relative fraction of unsaturated fatty acids in membranes. Unsaturated fatty acids solidify at lower temperatures than do saturated ones. Thus, the membranes retain their fluidity and function normally at cooler temperatures.

Low temperatures induce the formation of certain heat shock proteins that protect against chilling damage. There are also cases of "cross-protection" by heat shock proteins that are induced by one type of stress and that protect against other stresses. Tomatoes shocked by 2 days of high temperatures, for example, formed heat shock proteins and became resistant to chilling damage for the next 3 weeks.

If ice crystals form within cells, they can kill the cells by puncturing organelles and plasma membranes. Even outside cells, the growth of ice crystals can draw water from the cells and dehydrate them. Freezing-tolerant plants have a variety of adaptations to cope with these problems. A common one is the production of antifreeze proteins that inhibit the growth of ice crystals.

Plants have many effective mechanisms for coping with environmental challenges of many kinds. Their success is obvious—just look around you.

ra



Chapter Summary Plant-Pathogen Interactions

- ▶ Plants and pathogens evolve together. Review Figure 39.1
- ▶ Plants can strengthen their cell walls when attacked.
- ▶ Plant chemical defenses include PR proteins and phy-toalexins.
- ▶ In the hypersensitive response, cells produce phytoalexins and then die, trapping the pathogens in dead tissue.
- ▶ The hypersensitive response is often followed by systemic acquired resistance, in which the hormone salicylic acid activates further synthesis of PR proteins and triggers responses in other parts of the plant.
- ▶ The hypersensitive response is nonspecific. A more specific response, called gene-for-gene resistance, matches up alleles in a plant's resistance genes and a pathogen's avirulence genes. Review Figure 39.3

Plants and Herbivores: Benefits and Losses

- ▶ Grazing by herbivores increases the productivity of some plants. Review Figure 39.5
- ▶ Some plants produce secondary products that function as chemical defenses against herbivores. Review Table 39.1
- ▶ Various hormones, including systemin and jasmonates, participate in the pathways leading to the production of defensive chemicals. Review Figure 39.6
- ▶ To avoid poisoning themselves, plants may confine the toxic substances they produce to special compartments, or they may produce the substances only after cells have been damaged, or they may form enzymes and receptors that are not affected by the substances.

Water Extremes: Dry Soils and Saturated Soils

- ▶ Desert annuals evade drought by living only long enough to take advantage of the brief period during which the soil has enough moisture to support them.
- ▶ Some leaves have special adaptations to dry environments: a thickened cuticle, epidermal hairs, sunken stomata, fleshy leaves and stems, spines, and altered leaf display angles.
- ▶ Other adaptations to dry environments include long taproots and root systems that die back seasonally.
- ▶ The submerged roots of some plants form pneumatophores to allow oxygen uptake from the air. Aerenchyma in submerged plant parts stores and permits the diffusion of oxygen. Review Figure 39.14

Too Much Salt: Saline Environments

► A saline environment restricts the availability of water to plants. Halophytes are plants that are adapted to such environments.

► Most halophytes accumulate salt, and some have salt glands that excrete the salt to the leaf surface.

► Halophytes and xerophytes have some adaptations in common.

Habitats Laden with Heavy Metals

► Aluminum, mercury, lead, and cadmium are among the heavy metals that are toxic to plants at high concentrations.

► Rather than excluding heavy metals, tolerant plants deal with them after taking them up. A given plant's tolerance is limited to only one or two heavy metals.

Hot and Cold Environments

► High temperatures destabilize cell membranes and some proteins.

► Adaptations to elevated temperatures include the production of heat shock proteins.

► Low temperatures cause membranes to lose their fluidity.

PLANT RESPONSES TO ENVIRONMENTAL CHALLENGES 691

► Ice crystals can puncture organelles and plasma membranes.

► Adaptations to low temperatures and freezing include a change in membrane fatty acid composition and the production of antifreeze proteins.

For Discussion

1. We mentioned the possibility of designing crop plants that produce their own pesticides. Now chemical companies are designing crop plants capable of detoxifying

weed killers, so that crops grow after farmers have destroyed competing vegetation. Discuss the likely usefulness and possible drawbacks of such applications of recombinant DNA technology.

2. How might plant adaptations affect the evolution of herbivores? How might adaptations of herbivores affect plant evolution?

The stomata of the common oleander, *Nerium oleander*, are sunk in crypts in its leaves. Whether or not you know what an oleander is, you should be able to describe an important feature of its natural habitat; what is this feature?

Explain why halophytes often use the same mechanisms for coping with their challenging environments as xerophytes do for coping with theirs.

In ancient times, people used less sophisticated methods for mining than we use today. Thus ancient mines often yield substantial profits to modern-day miners who find and work them. On the basis of material in this chapter, how might you try to locate the site of an ancient mine?

Part Six

The Biology

of Animals



Physiology, Homeostasis, and Temperature Regulation

4fc r The camel is called the "ship of the

W[^]BP desert" because it can carry a large load across It 1 1 a hot desert without having to drink for days. / I i L Under similar conditions, a human sweats profusely to keep body temperature from rising, and can lose from 1 to 4 liters of water an hour. Without water, a human can become dangerously dehydrated in an hour. The dehydration causes the circulatory system and the thermoregulatory systems to fail, and body temperature begins to rise. As body temperature rises above 40°C, a person becomes dizzy and disoriented and gradually becomes delirious, loses consciousness, suffers brain damage, and dies. A person without water can die in the desert in only a few hours.

Why are camels better able to deal with desert conditions than we are? Several adaptations are important, but one of the most significant is that the camel's body temperature rises and falls more than ours does. Whereas humans try to keep their body temperatures close to 37°C by sweating, the camel allows its body temperature to rise to about 41°C over the course of the day. The camel's insulating coat of fur helps to slow its uptake of heat, but by tolerating the rise in its body temperature, it does not squander its body water for evaporative cooling.

When the air temperature falls at night, the camel passively unloads its accumulated heat to the environment. But now the situation reverses, and the camel allows its body temperature to fall. A human starts to produce heat by shivering when body temperature falls below about 36°C, but the camel's body temperature can fall below 34°C without stimulating shivering. The heat a camel stores in its body by allowing its body temperature to rise 7°C between sunup and sundown would require the evaporation of at least 5 liters of water to dissipate if the camel tried to maintain a constant body temperature. The camel has other adaptations, too. When it does reach water, it can drink about a third of its body weight to replace its losses!

The camel is an example of an animal that can live in an extreme environment. Physiology is the study of how organisms work—the study of the functions of all of the parts and processes of living systems. By studying the physiology of animals, we can understand how many species man-

Ships of the Desert

Camels conserve body water by allowing their body temperature to rise during the day rather than using evaporative cooling such as sweating and panting.

age to live in extreme environments. By studying the special adaptations of such species, we frequently gain new information about how the human body works.

Animal physiology is the focus of the next twelve chapters of this book. Each chapter will present a system of structures that provides essential functions for the animal body. A structure might be a single cell, or it might be a population of similar cells that make up a tissue, or it might be a collection of different kinds of tissues that make up an organ. Two or more interacting organs constitute an organ system that serves one or more physiological functions. Organs and organ systems function to maintain the various physical and chemical aspects of an animal's internal environment at optimal levels.



694 CHAPTER FORTY

In this chapter we set the stage for our study of animal physiology by presenting an overview of how cells are organized into tissues, tissues into organs, and organs into organ systems with different physiological functions. We discuss general principles of how organ systems are controlled and regulated to achieve constancy in the internal environment. Most of this chapter deals with one feature of the internal environment: temperature. We will see how temperature influences living systems, what adaptations animals have for dealing with temperature challenges, and finally, how mammals regulate body

temperature.

Homeostasis: Maintaining the Internal Environment

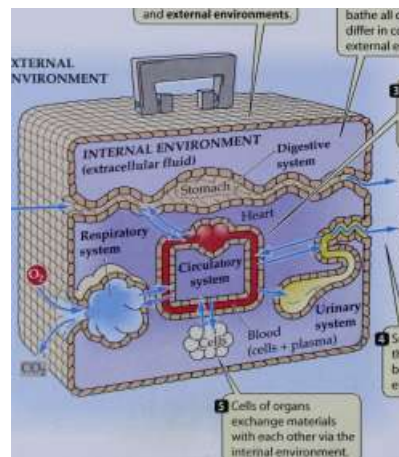
Single-celled organisms meet all their needs by direct exchanges with the external environment. Even the cells of some small, simple multicellular animals meet their needs in this way. Such animals are common in the sea. Seawater contains nutrients and salts, and provides a relatively unchanging physical environment. Most cells of a sponge or a jellyfish are in direct contact with seawater, or are close enough that they can receive nutrients and eliminate wastes without specialized organs to transport nutrients and wastes around their bodies. This lifestyle is quite limiting, however. No part of the animal's body can be more than a few cell layers thick, every cell must be able to take care of all its own needs, and the animal is limited to environments that provide for all of its cellular needs.

t

Skin separates the internal and external environments.

EXTERNAL ENVIRONMENT

Foods, salts, and water



The evolution of an internal environment, distinct from the external environment, made complex multicellular animals possible. The internal environment consists of extracellular fluids that bathe every cell of the body, supplying nutrients and receiving wastes. Its physical and chemical conditions can be kept at levels favorable to the cells. The cells are thereby protected from the external environment, making it possible for an animal to occupy habitats that would not support its cells if they were exposed to it directly. In complex multicellular organisms, it became possible for cells to become specialized for tasks that could contribute to maintaining specific aspects of the internal environment. Some cells became organized into tissues specialized to maintain the salt and water balance of the internal environment, others became specialized to provide nutrients, and still others to maintain appropriate levels of oxygen and carbon dioxide. Specialized tissues and organs form systems within the internal environment, each providing something all the cells of an animal need (Figure 40.1).

The composition of the internal environment is constantly being perturbed by the external environment and by the activities of cells themselves. The internal environment of a person in a desert, for example, will either increase in temperature or decrease in volume and change in composition because of water loss. Simultaneously, the activities of the person's cells will be taking nutrients from and contributing wastes to the internal environment. The activities of the specialized tissues and organs must continuously correct the physical and chemical composition of the internal environment so that it remains

conducive to life.

The maintenance of constant conditions in the internal environment is called homeostasis. Homeostasis is an essential feature of complex animals. If an organ fails to function properly, homeostasis is compromised, and as a result, cells become damaged and die. The damaged cells are not just those of the organ that functions improperly, but the cells of other organs as well. Loss of homeostasis is a serious problem that makes itself worse. To avoid loss of homeostasis, the activities of organs must be controlled and regulated in response to changes in both the external and the internal environments.

§j Fluids of the internal environment bathe all cells of the organism and differ in composition from the external environment.

A circulatory system moves materials to and from all parts of the internal environment.

Unabsorbed matter

Organic waste products, salts, and water

Some organs carry out the exchange of materials between the internal and external environments.

Cells of organs exchange materials with each other via the internal environment.

40.1 Maintaining Internal Stability While On the Go

Organ systems maintain a constant internal environment that provides for the needs of all cells of the body, making it possible for animals to travel among different and often highly variable external environments.

Control and regulation require information; hence the organ systems of information—the endocrine and nervous systems—must be included in our discussions of every physiological function. For that reason, we treat the endocrine and nervous systems early in this part of the book. Subsequent chapters deal with the systems responsible for controlling various aspects of the internal environment. Although each chapter will focus on different organs, those organs are all made of the same tissue types. What are these tissue types, and what are their general features?

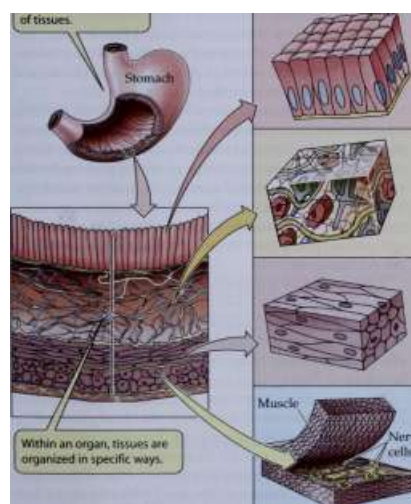
Tissues, Organs, and Organ Systems

Cells are the basic building blocks of multicellular animals. When cells with the same characteristics or specializations are grouped together, they form a tissue. There are four basic types of tissues—epithelial, connective, muscle, and nervous—but there are variations on each basic type. An organ is usually made up of several different tissue types (Figure 40.2).

Epithelial tissues cover the body and line organs

Epithelial tissues are sheets of densely packed, tightly connected cells that cover inner and outer body surfaces. They line hollow organs of the body such as the gut, the lungs, the bladder, and the blood vessels. Some epithelial cells have se-

An organ is composed of tissues.



Within an organ, tissues are organized in specific ways.

cretory functions—for example, those that secrete milk, mucus, digestive enzymes, or sweat. Others have cilia and help substances move over surfaces or through tubes. Since epithelial cells create boundaries between the inside and the outside of the body and between body compartments, they frequently have absorptive and transport functions. Epithelial cells can also be receptors that provide information to the nervous system. Smell and taste receptors, for example, are epithelial cells that detect specific chemicals.

An epithelial tissue can be classified according to its structure and the appearance of its cells:

► A simple epithelium is a single layer of cells, such as that forming the tubules of the kidney (Figure 40.3a).

► A stratified epithelium consists of multiple layers of cells, as is the case with the skin (Figure 40.3b).

► A pseudostratified epithelium really consists of a single layer of cells, but because the cells are of different lengths, they give the appearance of multiple layers (Figure 40.3c).

The shapes of the cells making up an epithelium can be squamous (flattened), cuboidal, or columnar. Most cuboidal and columnar epithelia are involved in transport or secretory functions and have an abundance of organelles such as mitochondria and Golgi apparatus. Squamous epithelia, such as the outer layer of the skin, have fewer organelles; they frequently serve structural functions and as permeability boundaries. Epithelial tissues have distinct inner and outer surfaces. The outer surface faces the air, as in the case of the skin and lungs, or a fluid-filled organ cavity, such as the lumen of the gut. These outer surfaces are made up of the apical ends of the epithelial cells, which may have cilia or may be highly folded to increase their surface area. The inner surface of an epithelium consists of the basal ends of the epithelial cells, which rest on an extracellular matrix called a basal lamina (see Figure 4.30).

The skin and the lining of the gut are examples of epithelial tissues that receive much wear and tear. Accordingly, cells in these tissues have a high rate of cell division to replace cells that die and are shed. Dandruff consists of discarded skin cells, and the well-known Pap smear test for cancer of the female reproductive tract is based on examination of shed epithelial cells.

Tissue type and function

Epithelial tissue

Lining, transport, secretion, and absorption

Connective tissue

Support, strength, and elasticity

Muscle tissue

Movement

Nervous tissue

r Nerve Information

cells synthesis,

communication, and control

40.2 Four Types of Tissue

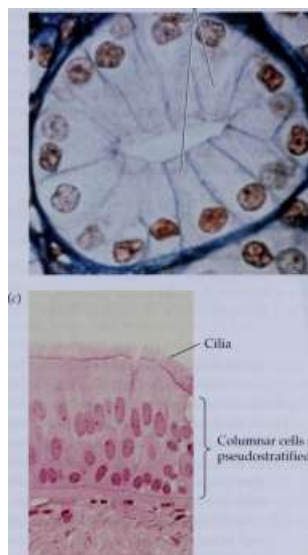
All cells can be classified into one of four tissue types. The cells of a given type have a similar structure and function.

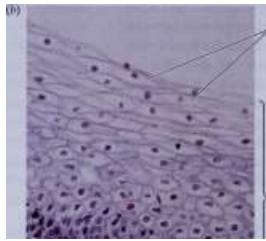
696 CHAPTER FORTY

(«)

Cuboidal cells

in simple epithelium





Squamous cells

Stratified epithelium

40.3 Epithelial Tissue

(a) A single layer of cuboidal cells forms a simple epithelium lining the collecting ducts of a human kidney, (b) Multiple layers of squamous cells form a stratified epithelium, (c) The columnar cells of this pseudostratified epithelium lining the respiratory tract give the appearance of multiple layers.

Columnar cells in pseudostratified epithelium

Connective tissues support and reinforce other tissues

In contrast to the densely packed epithelial tissues, connective tissues consist of dispersed populations of cells embedded in an extracellular matrix that they secrete. The composition and properties of the matrix differ among types of connective tissues.

An important component of the extracellular matrix is protein fibers secreted by the connective tissue cells. The dominant protein in the extracellular matrix is collagen, which is, in fact, the most abundant protein in the body (representing 25 percent of total body protein). Collagen fibers have high tensile strength. They give the dense connective tissue of skin, tendons, and ligaments resistance to stretch. Similarly, the collagen fibers of reticular connective tissue provide a netlike framework for organs, giving them shape and structural strength. Loose connective tissue fills spaces between organs and has a low density of collagen fibers.

Another type of protein fiber in the extracellular matrix of connective tissues is the stretchable protein elastin. It can be stretched to several times its resting length and then recoil. Fibers composed of elastin are most abundant in elastic

connective tissue, such as that in the walls of the lungs and the large arteries. Elastin fibers in the skin are responsible for its ability to snap back when stretched, and gradual loss of these fibers with age causes gradual loss of the resiliency of the skin.

Proteoglycans are extracellular proteins that give connective tissues resistance to compression. Proteoglycans are abundant in the extracellular matrix of the connective tissues lining joints.

Cartilage and bone are connective tissues that provide rigid structural support. In cartilage, a network of collagen fibers is embedded in a rather flexible matrix consisting of a protein-carbohydrate complex called chondroitin sulfate. The cells that form cartilage are called chondrocytes, and they exist in small cavities in the cartilage (Figure 40.4a). Cartilage forms the entire skeletal system of sharks and rays, which are therefore called cartilaginous fishes.

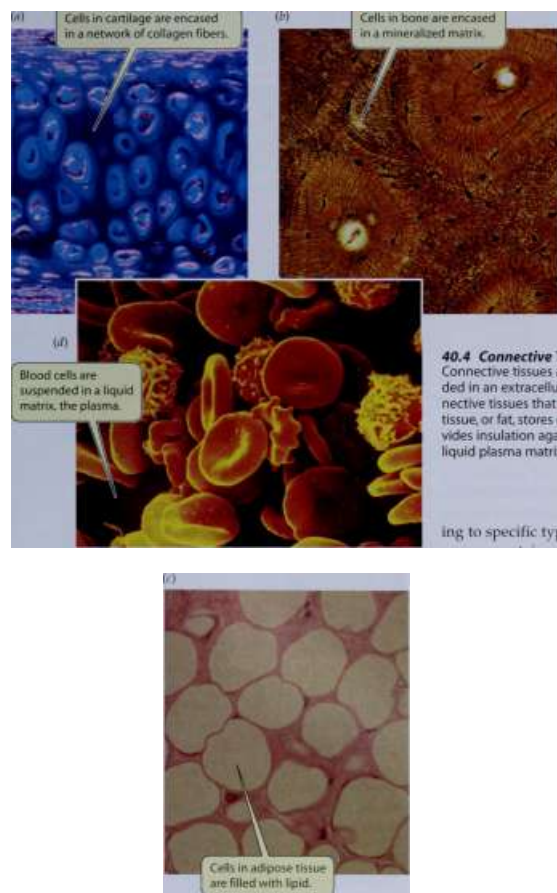
Cartilage forms the skeletons of the early developmental stages of more complex vertebrates, but it is gradually replaced by bone, a harder connective tissue (Figure 40.4b). Adult vertebrates retain cartilage as the support for flexible structures such as external ears, noses, and the windpipe. The extracellular matrix in bone also contains many collagen fibers, but it is hardened by the deposition of the mineral calcium phosphate. We will discuss bone in greater detail in Chapter 44.

Adipose tissue is a form of loose connective tissue that includes adipose cells, which form and store droplets of lipids (Figure 40.4c). Adipose tissue is a major source of stored energy, but it also serves to cushion organs, and layers of adipose tissue under the skin can provide a barrier to heat loss.

Blood is a connective tissue consisting of cells dispersed in an extensive extracellular matrix: the blood plasma (Figure 40.4d). The blood plasma is much more liquid than the extracellular matrices of the other connective tissues, but it too contains an abundance of proteins. One of those proteins, fibrinogen, serves a structural function when it is stimulated to polymerize and form a blood clot. Many of

Cells in cartilage are encased in a network of collagen fibers.

Cells in bone are encased in a mineralized matrix.



Blood cells are suspended in a liquid matrix, the plasma.

the proteins and cellular elements of the blood were presented in Chapter 19, and blood will be discussed again in Chapter 49.

Muscle tissues contract

Muscle tissues consist of elongated cells that can contract and cause movement. Muscle tissues are the most abundant tissues in the body, and they use most of the energy produced in the body. The contraction of muscle cells depends on intracellular protein filaments that can slide past each other. In Chapter 47, we will encounter three types of muscle tissues:

- Skeletal muscle connects bones to bones and is responsible for the body movements that constitute behavior.
- Smooth muscle is found in internal organs and is not under voluntary control; it performs functions such as moving food through the gut and constricting blood vessels.
- Cardiac muscle makes up the mass of the heart and pumps the blood.

Nervous tissues process information

There are two basic cell types in nervous tissues: neurons and glial cells. [Neurons, which are extremely diverse in size and form, generate electrochemical signals. Respond-

40.4 Connective Tissues

Connective tissues are of dispersed populations of cells embedded in an extracellular matrix, (a) Cartilage and (b) bone are connective tissues that provide rigid structural support, (c) Adipose tissue, or fat, stores energy, cushions the internal organs, and provides insulation against the cold, (d) Red and white blood cells in a liquid plasma matrix make up the connective tissue blood.

ing to specific types of stimuli, such as light, sound, pressure, or certain molecules, neurons generate sudden voltage changes across their plasma membranes. These nerve impulses can be conducted via long extensions of the neurons to other parts of the body, where they are communicated to other neurons, muscle cells, or secretory cells. Neurons are involved in controlling the activities of most organ systems to achieve homeostasis.

Glial cells do not generate or conduct electric signals, but they provide a variety of supporting functions for neurons. There are more glial cells than neurons in our nervous systems. We will detail and illustrate the properties of nervous

tissues in Chapters 44, 45, and 46.

Organs consist of multiple tissues

A discrete structure that carries out a specific function in the body is called an organ. Examples are the stomach, the heart,

the liver, or the kidney. Most organs include all four tissue types. The wall of the stomach is a good example (see Figure 40.2). The inner surface of the stomach that contacts food is lined with a simple epithelium. Some of the epithelial cells secrete mucus, enzymes, or stomach acid.

Beneath the epithelial lining is connective tissue. Within this connective tissue are nerves, glands (secretory epithelial cells), and blood vessels. Concentric layers of muscle tissue enable the stomach to contract to mix food with the digestive juices. A network of neurons between the muscle layers controls these movements and also partially controls the secretions of the stomach. Surrounding the stomach is a layer of connective tissue called the serosa.

698 CHAPTER FORTY

Skeletal system Reproductive system

Digestive system

Gas exchange system Circulatory system

Lymphatic system

Immune system Skin system

Excretory system

Bones

Female: ovaries, oviducts, uterus, vagina, mammary glands Male: testes, sperm ducts, accessory glands, penis

Mouth, esophagus, stomach, intestines, liver, pancreas, rectum, anus

Airways, lungs, diaphragm

Heart and blood vessels

Lymph and lymph vessels, lymph nodes, spleen

Many types of white blood cells Skin, sweat glands, hair

Kidneys, bladder, ureter, urethra

Provides structural support for the body. Chapter 47

Produces sex cells and hormones necessary to procreate and nurture offspring. Chapter 42

Acquires and digests food, absorbs and stores nutrients, then makes them available to the cells of the body. Chapter 50

Exchanges respiratory gases with the environment. Chapter 48

Transports respiratory gases, nutrients, hormones, and heat around the body. Chapter 49

Brings extracellular fluids back into the circulatory system; helps the immune system fight invading organisms. Chapters 40 and 19

Fights invading organisms and infections. Chapter 19

Protects the body from invading organisms and harsh physical conditions, helps regulate body temperature. Chapter 40

Regulates the composition of the extracellular fluids; excretes waste products. Chapter 51

An individual organ is usually part of an organ system—a group of organs that function together. The stomach is part of the digestive system, which also includes the food tube (esophagus), the small and large intestines, the pancreas, which secretes digestive enzymes, and the liver, which secretes bile. The major organ systems of mammals are outlined in Table 40.1.

^ Physiological Regulation and Homeostasis

Homeostasis depends on the ability to regulate the functions of the organs and organ systems to counteract influences that would change the physical or chemical composition of the internal environment. In this section we discuss the general properties of physiological regulatory systems, and then consider temperature regulation as a specific example.

Set points and feedback information are required for regulation

In addition to control mechanisms, regulation requires information. You can regulate the speed of a car only if you

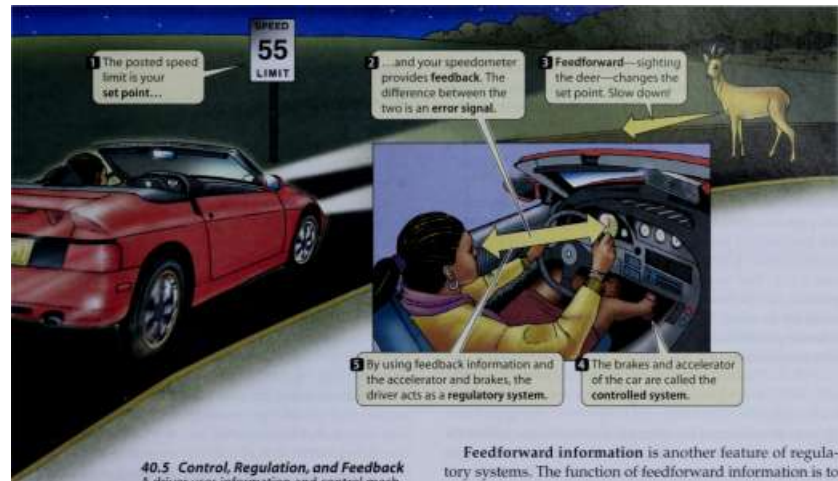


know the speed at which you are traveling and the speed you wish to maintain. The desired speed is a set point, and the

reading on your speedometer is feedback information. When the set point and the feedback are compared, any difference between them is an error signal. Error signals suggest corrective actions, which you make by using the accelerator or brake (Figure 40.5).

Physiological regulation requires actions of cells, tissues, and organs, which are called effectors because they effect changes. Effectors are also referred to as controlled systems because their activities are controlled by commands coming from regulatory systems. Regulatory systems obtain, process, and integrate information and issue commands to the controlled systems. A fundamental way to analyze a regulatory system is to identify its source of feedback information.

Negative feedback is the most common type of feedback information in regulatory systems. The word "negative" indicates that this feedback information causes the effectors to reduce or reverse the process or influence that created the signal. In our car analogy, the recognition that you are going too fast is negative feedback if it causes you to slow down.



40.5 Control, Regulation, and Feedback

A driver uses information and control mechanisms to regulate the speed of the car.

Thermostats regulate temperature

The thermostat that is part of the heating-cooling system of a house is a regulatory system. It has upper and lower set points that you can adjust, and it receives feedback information from a sensor that measures room temperature. The circuitry of the thermostat converts differences between the set points and feedback information into signals that activate the controlled systems—the furnace and the air conditioner.

When room temperature rises above the upper set point, the thermostat activates the air conditioner to reduce the temperature; when room temperature falls below that upper set point, the air conditioner is turned off. If temperature falls below the lower set point, the furnace is activated, raising room temperature. The mechanism that senses room temperature provides negative feedback that is used to regulate both the air conditioner and the furnace. Negative feedback is a stabilizing influence in physiological regulatory systems. It contributes to homeostasis by stimulating actions that return a variable to its set point.

Is there any such thing as positive feedback in physiology? Although not as common as negative feedback, it does exist. Rather than returning a system to a set point, positive feedback amplifies a response. Examples of regulatory systems that use positive feedback are the responses that empty body cavities, such as urination, defecation, sneezing, and vomiting. Another example is sexual behavior, in which a little stimulation causes more behavior, which causes more stimulation, and so on.

Feedforward information is another feature of regulatory systems. The function of feedforward information is to change the set point. Seeing a deer ahead on the road when you are driving is an example of feedforward information (see Figure 40.5); this information takes precedence over the posted speed limit, and you change your set point to a slower speed. If you want the temperature of your house to be lower at night than during the day, you can add a clock to the thermostat to provide feedforward information about time of day.

These principles of control and regulation help organize our thinking about physiological systems. Once we understand how an organ or an organ system works, we can then ask how is it regulated. As an example, we will discuss in detail the system that regulates body temperature. But first, why is it necessary to regulate body temperature?

Temperature and Life

Over the face of Earth, temperatures vary enormously, from the boiling hot springs of Yellowstone National Park to the interior of Antarctica, where the temperature can fall below -80°C . Because heat always moves from a warmer to a cooler object, any change in the temperature of the environment causes a change in the temperature of an organism in that environment—unless the organism does something to regulate its temperature.

Living cells function over only a narrow range of temperatures. If cells cool to below 0°C , ice crystals damage their structures, possibly fatally. Some animals have adaptations such as antifreeze molecules in their blood that help them resist

freezing; others have adaptations that enable them to survive freezing. Generally, however, cells must remain above 0°C to stay alive.

700 CHAPTER FORTY

The upper temperature limit is less than 45°C for most cells. Some specialized algae can grow in hot springs at 70°C, and some archaea can live at near 100°C, but in general, proteins begin to denature and lose their function as temperatures approach 45°C. Most cellular functions are limited to the range between 0°C and 45°C, which are considered the thermal limits for life. A particular species, however, generally has much narrower limits.

Q_{10} is a measure of temperature sensitivity

Within the range 0° to 45°C / temperature changes create problems for animals. Most physiological processes, like the biochemical reactions that constitute them, are temperature-sensitive, going faster at higher temperatures (see Figure 6.26). The temperature sensitivity of a reaction or process can be described in terms of Q_{10} , a quotient calculated by dividing the rate of a process or reaction at a certain temperature, R_T , by the rate of that process or reaction at a temperature 10°C lower, R_{T-10} :

R_T

Q_k

Q_{10} can be measured for a simple enzymatic reaction or for a complex physiological process, such as the rate of oxygen consumption. If a reaction or process is not temperature-sensitive, it has a Q_{10} of 1. Most biological Q_{10} values are between 2 and 3, which means that reaction rates double or triple as temperature increases by 10°C (Figure 40.6).

Changes in temperature can be particularly disruptive to an animal's functioning because all the component reactions in the animal do not have the same Q_{10} . Individual reactions with different Q_{10} 's are linked together in complex networks that carry out physiological processes. Changes in temperature shift the rates of some reactions

11

10

9

•2 7

a 6

* 5

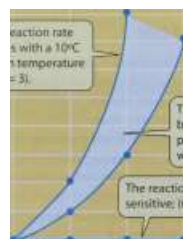
DC

J A o :

v r-io



The reaction rate triples with a 10°C rise in temperature ($Q_{10} = 3$).



The reaction rate doubles with a 10°C rise in temperature ($Q_{10} = 2$).

The rates of most biochemical reactions and physiological processes fall within this range.

ion is not temperature-sensitive ($Q_{10} = 1$)

10 20 30 40

Temperature (°C)

50

60

40.6 Q₁₀ and Reaction Rate

The larger the Q_{10} , the faster the reaction rate rises in response to an increase in temperature.

more than those of others, thus disrupting the balance and integration that the processes require. For homeostasis, organisms must be able to compensate for or prevent changes in temperature.

An animal's sensitivity to temperature can change

The body temperature of some animals is tightly coupled to the environmental temperature. Think of a fish in a temperate-zone pond. As the temperature of the pond changes from 4°C in midwinter to 24°C in midsummer, the body temperature of the fish does the same (Figure 40.7). We can bring the fish into the laboratory in the summer and measure its metabolic rate (the sum total of the energy turnover of its cells, often measured by O_2 consumption). If we measure the metabolic rate at different water temperatures, we might plot our data as shown by the red line in Figure 40.7 and calculate a Q_{10} of 2. We predict from our graph that in winter, when the temperature is 4°C, the fish's metabolic rate will be only one-fourth of what it was in the summer. We then return the fish to its pond.

When we bring the fish back to the laboratory in the winter and repeat the measurements, we find, as the blue line shows, that its metabolic rate at 4°C is not as low as we predicted; rather, it is almost the same as it was at 24°C in the summer. If we repeat the measurement over a range of temperatures, we find that the fish's metabolic rate is always higher than the rate we predicted from the measurement we took at the same temperature in the summer. This difference is due to acclimatization, the process of physiological and biochemical change that an animal undergoes in response to seasonal changes in climate.

Seasonal acclimatization in the fish has produced metabolic compensation, which readjusts the biochemical machinery to counter the effects of temperature. What might account for such a change? Look again at Figure 6.26, which shows the different optimal temperatures of enzymes. If the fish can express similar enzymes that operate at different optimal temperatures, it can compensate metabolically by catalyzing reactions with one set of enzymes in summer and another set in winter. The end result is that metabolic functions are much less sensitive to long-term changes in temperature than they are to short-term thermal fluctuations.

Maintaining Optimal Body Temperature

Animals can be classified by how they respond to environmental temperatures.

- A homeotherm is an animal that maintains a constant body temperature.
- A poikilotherm is an animal whose body temperature changes when the temperature of its environment (the ambient temperature) changes.

This system of classification says something about the biology of the animals, but it presents problems. Should a fish in the deep ocean, where the temperature changes very little, be called a homeotherm? Should a hibernating mam-

EXPERIMENT

Question: How does a fish acclimatize to seasonal temperature changes?

METHOD Bring fish into the lab—in winter and summer—and measure its metabolic rate (O_2 consumption) at its natural pond temperature (it) and at other temperatures (•).

RESULTS

O_2
4°C
Winter pond temperature
14°C
24°C
Summer pond temperature

Conclusion: Between summer and winter a slow process of acclimatization compensates for seasonal temperature changes.

40.7 Metabolic Compensation

In its natural environment, a fish's metabolism readjusts, or acclimatizes, to compensate for seasonal changes in temperature. regulates its body temperature at a constant level some of the time.

Another set of terms classifies animals on the basis of the sources of heat that determine their body temperatures.

- Ectotherms depend largely on external sources of heat, such as solar radiation, to maintain their body temperatures above

the environmental temperature.

► Endotherms can regulate their body temperatures by producing heat metabolically or by mobilizing active mechanisms of heat loss.

Mammals and birds are endotherms; animals of all other species behave as ectotherms most of the time.

Ectotherms and endotherms respond differently in metabolic chambers

A small lizard is an example of an ectotherm. We can compare it with a mouse, which is an endotherm of the same body size. We can put each animal in a metabolic chamber and measure body temperatures and metabolic rates as we change the temperature of the chamber from 0°C to 35°C.

The results obtained from the two species differ. The body temperature of the lizard equilibrates with that of the chamber, whereas the body temperature of the mouse remains at 37°C (Figure 40.8#). The metabolic rate of the lizard decreases as the temperature decreases (Figure 40.8b). In contrast, the mouse's metabolic rate increases as chamber temperature falls below about 27°C (notice that you must read the graph right to left to see this). The lizard apparently cannot regulate its body temperature or metabolism independently of environmental temperature. The mouse, however, regulates its body temperature by in-

mal that allows its body temperature to drop to nearly the temperature of its environment be called a poikilotherm? The problem posed by the hibernator has been solved by creating a third category: the heterotherm, an animal that

40.8 Ectotherms and Endotherms

The body temperatures of a lizard and a mouse of the same body size respond differently to changes in environmental temperature.

(«)

U

01

Pl,

O

40 -



30-

20-

10-

t

The body temperature of an endotherm remains constant...

...while that of an ectotherm fluctuates with the environmental temperature.



10 20 30

Environmental temperature (°C)

37 40

(b)

c

T₃

O

rn

| As the environmental temperature rises, metabolic heat production decreases in endotherms...

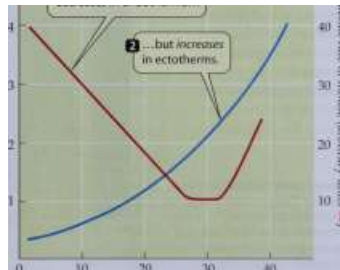
Notice the difference in the two scales. At all temperatures, the metabolism of the lizard is much less than that of the mouse.

10 20 30 40

Environmental temperature (°C)

50

y



702 CHAPTER FORTY

creasing its metabolic rate, which increases its production of body heat.

Ectotherms and endotherms use behavior to regulate body temperature

We can test our laboratory conclusion that the lizard cannot regulate its body temperature. To do this, we release the lizard in its desert habitat and measure its temperature as it goes about its normal behavior in this environment, where temperature can change 40°C in a few hours (Figure 40.9).

Unlike what we observed in the metabolic chamber, the body temperature of the lizard is at times considerably different from the environmental temperature. At night, the temperature in the desert may drop close to freezing, but the temperature of the lizard remains stable at 16°C. No mystery here: The lizard spends the night in a burrow, where the soil temperature is a constant 16°C.

Early in the morning, soon after sunrise, the lizard emerges from its burrow. The air temperature is still cool, but the lizard's body temperature rises above 30°C in less than 30 minutes. The lizard achieves this by basking on a rock with maximum exposure to the sun. As its skin absorbs solar radiation, its body temperature rises considerably above the air temperature. By altering its exposure to the sun, the lizard maintains its body temperature around 35°C all morning.

By noon, the air temperature near the surface of the desert has risen to 50°C, but the lizard maintains its body temperature around 35°C by staying mostly in the shade and frequently in the branches of bushes, where there is a cooling breeze. As afternoon progresses, the air cools, and the lizard again spends more of its time in the sun and on hot rocks to maintain its body temperature around 35°C. The lizard returns to its burrow just before sunset, and its body temperature rapidly drops to 16°C.

Our conclusion must be that the lizard can regulate its body temperature quite well by behavioral mechanisms rather than by internal metabolic mechanisms. In our laboratory experiment, the lizard in the chamber could not use its thermoregulatory behavior, but in its natural environment it could move to different places to alter the heat exchange between its internal and external environments.

But behavioral thermoregulation is not the exclusive domain of ectotherms. It is also the first line of

40.9 An Ectotherm Uses Behavior to Regulate Its Body Temperature

The lizard's body temperature is dependent on environmental heat, but it can regulate its temperature by moving between different environments.

Sunrise

defense for endotherms. When the option is available, most animals select the thermal microenvironments that are best for them. They may change their posture, orient to the sun, move between sun and shade, and move between still air and moving air, as demonstrated by the lizard in our field experiment. Examples of more complex thermoregulatory behavior are nest construction and social behavior such as huddling. Humans select appropriate clothing and heat or cool their buildings. Behavioral thermoregulation is widespread in the animal kingdom (Figure 40.10).

Both ectotherms and endotherms control blood flow to the skin

Just as behavioral thermoregulation is not the exclusive domain of ectotherms, physiological thermoregulation is not the exclusive domain of endotherms. Both ectotherms and endotherms can alter the rate of heat exchange between their bodies

and their environments by controlling the flow of blood to the skin.

The skin is the interface between the internal and the external environment, and heat exchanges that alter body temperature occur across this interface. Heat exchange between the skin and the external environment occurs through four mechanisms: radiation, conduction, convection, and evaporation (Figure 40.11).

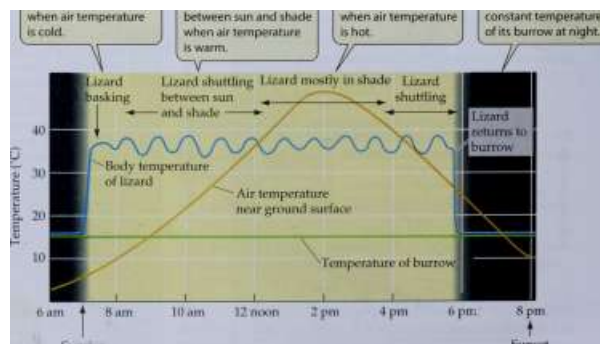
Heat exchange between the internal environment and the skin occurs largely through blood flow. For example, when a person's body temperature rises as a result of exercise, blood flow to the skin increases, and the skin surface becomes quite warm. The heat brought to the skin by the blood is lost to the environment, and this loss helps to bring the body temperature back to normal. In contrast, when a person is exposed to cold, the blood vessels supplying the skin constrict, decreasing blood flow and heat transport to the skin and reducing heat loss to the environment.

A lizard basks in the sun when air temperature is cold.

£] The lizard shuttles between sun and shade when air temperature is warm.

JJThe lizard avoids sun when air temperature is hot.

JJThe lizard returns to the constant temperature of its burrow at night.



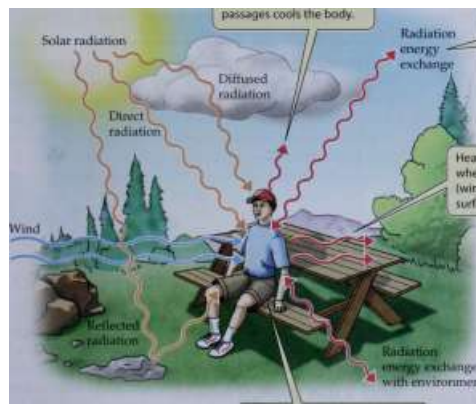
(b)



The control of blood flow to the skin can be an important adaptation for an ectotherm like the marine iguana of the Galapagos archipelago. The Galapagos are volcanic islands that lie on the equator, but they are bathed by very cold oceanic currents. Marine iguanas are reptiles that bask on black lava rocks on shore and swim in the cold ocean, where they feed on algae. When the iguanas are feeding,

Evaporation of water from body surfaces or breathing passages cools the body.

Solar radiation



40.70 Endotherms Can Use Behavior to Thermoregulate

(a) Humans must put on many layers of insulating clothing to help their thermoregulatory mechanisms keep pace with the extreme cold of western Siberia. (f<) When air temperatures on the African savanna soar, an elephant may use a cool shower to thermoregulate.

they cool to the temperature of the sea, which makes them slower and more vulnerable to predators, and probably incapable of efficient digestion. They therefore alternate between feeding in the cold

— sea and basking in the sun

on the hot rocks. It is advantageous for iguanas to retain body heat as long as possible while swirruring and to warm up as fast as possible when basking. They adjust by changing their heart rate, and therefore their blood flow (Figure 40.12).

Some ectotherms produce heat

Some ectotherms raise their body temperatures by producing heat. For example, the powerful flight muscles of many insects must reach $35^{\circ}\text{--}40^{\circ}\text{C}$ before the insects can fly, and they must maintain these high temperatures during flight,

even at air temperatures around 0°C . Such insects produce the required heat by contracting their flight muscles in a manner analogous to shivering in mammals (Figure 40.13). The heat-producing ability of these insects can be quite remarkable. Probably the most impressive case is a species of scarab beetle that lives mostly underground in mountains north of Los Angeles, California. To mate, these beetles come above ground, and males fly in search of females. They un-

Objects in the environment exchange radiation with each other and with the sky. Warmer objects lose heat to cooler objects.

Heat is lost by convection /hen a stream of air (wind) is below body surface temperature.

Radiation energy exchange with environment

Conduction is the direct transfer of heat when objects of different temperatures come into contact.

40.11 Animals Exchange Heat with the Environment

An animal's body temperature is determined by the balance between internal heat production and the avenues of heat exchange with the environment: radiation, conduction, convection, and evaporation.

704 CHAPTER FORTY

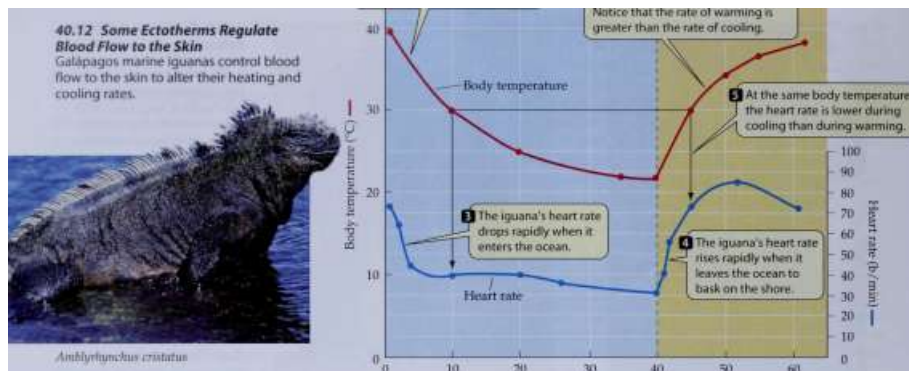
40.12 Some Ectotherms Regulate Blood Flow to the Skin

Galapagos marine iguanas control blood flow to the skin to alter their heating and cooling rates.

f

As soon as the iguana enters the ocean, it begins to cool.

When the iguana leaves the ocean to bask on the shore, it begins to warm. Notice that the rate of warming is greater than the rate of cooling.



Amblyrhynchus cristatus

undertake this mating ritual at night, in winter, and only during snowstorms.

Honeybees regulate temperature as a group. They live in large colonies consisting mostly of female worker bees that maintain the hive and rear the offspring of the single queen bee. During winter, honeybee workers combine their individual heat-producing abilities to regulate the temperature of the brood. They cluster around the brood and adjust their joint metabolic heat production and density of clustering so that the brood temperature remains remarkably constant, at about 34°C, even as the outside air temperature drops below freezing.

Some reptiles use metabolic heat production to raise their body temperatures above the air temperature. The female Indian python protects her eggs by coiling her body around them. If the air temperature falls, she contracts the muscles of her body wall to generate heat. The python is able to maintain the temperature of her body—and therefore that of her eggs—above air temperature.

Some fish elevate body temperature by conserving metabolic heat

It is particularly difficult for fish to raise their body temperatures because blood pumped from their hearts goes to the gills to pick up oxygen, where it comes into close contact with cool water flowing over the thin gill membranes. The blood equilibrates with the outside water temperature before it travels through the rest of the body. Any heat transferred to the blood from active muscles is lost rapidly to the environment when the blood flows through the gills. It is thus surprising to find that some large, rapidly swimming fishes, such as bluefin tuna and great white sharks, can maintain temperature differences as great as 10°-15°C between their bodies and the surrounding water. The heat comes from their powerful swimming muscles, and the ability of these "hot" fish to conserve that heat is due to remarkable arrangements of their blood vessels.

30 40

Time (min)

In the usual ("cold") fish circulatory system, oxygenated blood from the gills collects in a large dorsal vessel, the aorta, which travels through the center of the fish, distributing blood to all organs and muscles (Figure 40.14a). "Hot" fish have a smaller central dorsal aorta. Most of their oxygenated blood is transported in large vessels just under the skin (Figure 40.14b). Hence the cold blood from the gills is kept close to the surface of the fish. Smaller vessels trans-

During pre-flight warm-up, flight muscles contract simultaneously. Pulling against one another, they produce heat but do not cause the wings to beat (similar to human shivering).

40

35

U

5

CO (H

01

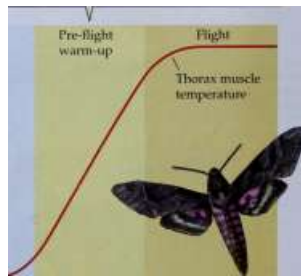
£. 30 S

01

H

25

Rest



-10 12 3 4

Time from onset of warm-up (min)

40.13 A Moth's Preflight Warm-Up

Before takeoff, insects such as the sphinx moth contract the flight muscles in the thorax to generate heat and warm the muscles up to the temperature required for flight. This mechanism enables the moth to fly even at night, when the environment is cool.

(fl)

(b)

"COLD" FISH

"HOT" FISH

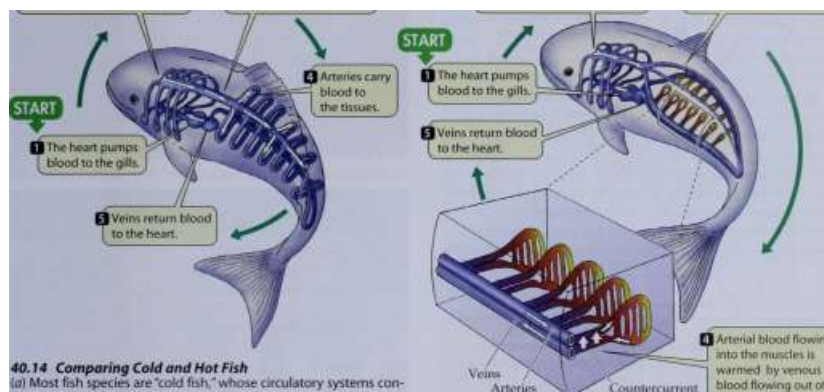
I In the gills, blood is oxygenated and comes to the same temperature as water.

I Cold blood flows through the center of the fish in the large dorsal aorta.

T

In the gills, blood is oxygenated and comes to the same temperature as water.

I Cold blood flows from the gills to the body in arteries just under the skin.



40.14 Comparing Cold and Hot Fish

(a) Most fish species are "cold fish," whose circulatory systems conduct cool, oxygenated blood from the gills through a large dorsal aorta to the rest of the body, (b) The blood vessel anatomy of "hot" fish species allows for heat exchange between the cold arterial blood entering the muscles and the departing venous blood, which has been warmed by the metabolism of the muscles.

porting this cold blood into the muscle mass run parallel to the vessels transporting warm blood from the muscle mass back toward the heart. Since the vessels carrying the cold blood into the muscle are in close contact with the vessels carrying warm blood away, heat flows from the warm to the cold blood and is therefore trapped in the muscle mass. Because heat is exchanged between blood vessels carrying blood in opposite directions, this adaptation is called a countercurrent heat exchanger. It keeps the heat within the muscle mass, enabling the fish to have an internal body temperature considerably above the water temperature. Why is it advantageous for the fish to be warm? Each 10°C rise in muscle temperature increases the fish's sustainable power output almost threefold!

Thermoregulation in Endotherms

As we saw in Figure 40.8, endotherms respond to changes in environmental temperature by changing their rates of heat production, measured as metabolic rate. Within a narrow range of environmental temperatures called the thermoneutral zone, the metabolic rate of endotherms is low and independent of temperature. The metabolic rate of a resting animal at a temperature within the thermoneutral zone is called the basal metabolic rate. It is usually measured on animals that are quiet but awake, and that are not

Countercurrent heat exchanger

I Arterial blood flowing into the muscles is warmed by venous blood flowing out of the muscles.

using energy for digestion, reproduction, or growth. A resting animal consumes energy at the basal metabolic rate just to carry out all of its minimal body functions.

The basal metabolic rate of an endotherm is about six times greater than the metabolic rate of an ectotherm of the same size and at the same body temperature (see Figure 40.8b). A gram of mouse tissue consumes energy at a much higher rate than does a gram of lizard tissue when both tissues are at 37°C. This difference results from basic changes in cell metabolism that accompanied the evolution of endotherms from their ectothermic ancestors.

Endotherms actively increase heat production or heat loss

The thermoneutral zone is bounded by a lower critical temperature and an upper critical temperature. Figure 40.15 describes the thermoregulatory responses of a mammal at temperatures outside its thermoneutral zone.

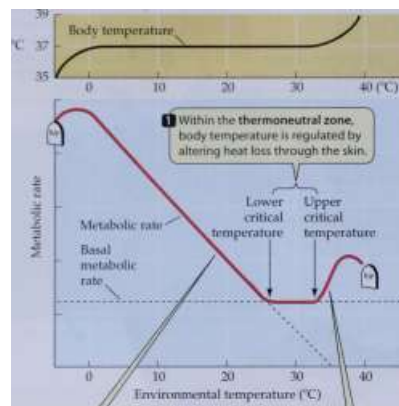
When the environmental temperature falls below the lower critical temperature, mammals can create heat for thermoregulation through shivering and nonshivering heat production. Birds use only shivering heat production. Shivering uses the contractile machinery of skeletal muscles to consume ATP without causing observable behavior. The muscles pull against each other so that little movement other than a tremor results. All the energy from the conversion of ATP to ADP in this process is released as heat. Shivering heat production is perhaps too narrow a term; energy

706 CHAPTER FORTY

Body temperature

40 (°C)

Q Within the thermoneutral zone, body temperature is regulated by altering heat loss through the skin.



Upper critical temperature

o Below the lower critical temperature, the animal produces metabolic heat to compensate for increased heat loss to the environment.

§) Above the upper critical temperature, the animal must expend energy to lose heat by panting or sweating, which makes its metabolic rate increase.

Vertebral column

Heart

expenditure due to increased muscle tone and increased body movements also contributes to increased heat production in the cold.

Most nonshivering heat production occurs in specialized adipose tissue called brown fat (Figure 40.16). This tissue looks brown because of its abundant mitochondria and rich blood supply. In brown fat cells, a protein called thermogenin uncouples proton movement from ATP production, allowing protons to leak across the inner mitochondrial membrane rather than having to pass through the ATP synthase protein and generate ATP (review the discussion of respiration in Chapter 7). As a result, metabolic fuels are consumed without producing ATP, but heat is still released. Brown fat is especially abundant in newborn infants of many mammalian species, in some adult mammals that are small and acclimatized to cold, and in mammals that hibernate.

Decreasing heat loss is important for life in the cold



Outside the thermoneutral zone, maintaining a constant body temperature requires the expenditure of energy.

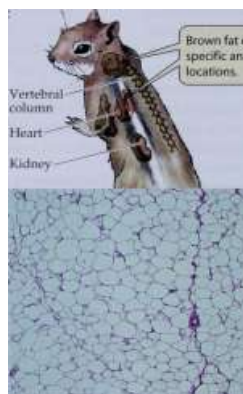
mammals, but almost no reptiles or amphibians, live in very cold habitats. What adaptations besides endothermy characterize species that live in the cold?

The most important adaptations of endotherms to cold environments are those that reduce heat loss to the environment. Since most heat is lost from the body surface, many cold-climate species have a smaller surface area than their warm-climate cousins, even when their body masses are the same. Rounder body shapes and shorter appendages reduce the surface area-to-volume ratios of some cold-climate species; compare, for example, the San Joaquin kit fox and the arctic fox (Figure 40.17).

Another means of decreasing heat loss is to increase thermal insulation. Animals adapted to cold have much thicker layers of fur, feathers, or fat than do their warm-climate relatives. The fur of an arctic fox or a northern sled dog provides such good thermal insulation that those animals don't even begin to shiver until the air temperature drops as low as -20°C to -30°C .

Fur and feathers are good insulators because they trap a layer of still, warm air close to the skin surface. If that air is displaced by water, insulation is drastically reduced. In many species, oil secretions spread through fur or feathers by grooming are critical for resisting wetting and maintain-

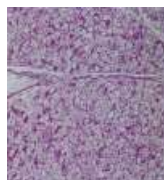
Brown fat occurs in specific anatomical locations.



40.76 Brown Fat

In many mammals, specialized brown fat tissue produces heat.

;;w^-



The coldest habitats on Earth are in the Arctic, the Antarctic, and at the peaks of high mountains. Many birds and

White fat viewed through a light microscope. Each cell is filled with a globule of lipid and has few organelles. The tissue has few blood vessels.

Brown fat viewed through a light microscope at the same magnification reveals cells with many intracellular structures and multiple droplets of lipid.



(a) *Vulpes macrotis*



(b) *Lopex lagopus*

40.17 Adaptations to Hot and Cold Climates

(a) The San Joaquin kit fox, a desert dweller, has a large surface area for its body. The large ears serve as heat exchangers, passing heat from the fox's blood to the surrounding air. (f>) The thick fur of the arctic fox provides insulation in the frigid winter. Its ears and extremities are relatively smaller than those of the kit fox.

ing a high level of insulation.

Humans change their thermal insulation by putting on or taking off clothes. How do other animals do it? We have already discussed one example, the ectothermic marine iguana, which controls blood flow to its skin. Increasing or decreasing blood flow to the skin is an important thermoregulatory adaptation for endotherms as well. In a hot environment, your skin feels hot because there is a high rate of blood flow through it, but when you sit in an overly airconditioned theater, your hands, feet, and other body surfaces feel cold as blood flow to those areas decreases.

Evaporation of water is an effective way to lose heat

For highly insulated arctic animals, and for many large mammals in all climates, getting rid of excess heat can be a serious problem, especially during exercise. Arctic species usually have an area on the body surface, such as the abdomen, that has only a thin layer of fur and can act as a window for heat loss. Large mammals, such as elephants, rhinoceroses, and water buffalo, have little or no fur and seek places where they can wallow in water when the air

temperature is too high. Having water in contact with the skin greatly increases heat loss because water has a much greater capacity for absorbing heat than air does.

A gram of water absorbs about 580 calories of heat when it evaporates. Water is heavy, however, so animals do not carry an excess supply of it. Furthermore, hot environments tend to be arid places where water is a scarce resource. Therefore, evaporation of water by sweating or panting is usually a last resort for animals adapted to hot environments (recall the camels at the beginning of this chapter).

Sweating and panting are active processes that require the expenditure of metabolic energy. That's why the metabolic rate increases when the upper critical temperature is exceeded (see Figure 40.15). A sweating or panting animal is producing heat in the process of dissipating heat, which can be a losing battle. Animals can survive in environments that are below their lower critical temperature much better than they can in environments above their upper critical temperature.

The Vertebrate Thermostat

The thermoregulatory mechanisms and adaptations we have discussed are the controlled systems for the regulation of body temperature. These controlled systems must receive commands from a regulatory system that integrates information relevant to the regulation of body temperature. Such a regulatory system can be thought of as a thermostat. All animals that thermoregulate, both vertebrate and invertebrate, must have regulatory systems, but here we will focus on the vertebrate thermostat.

Where is the vertebrate thermostat? Its major integrative center is at the bottom of the brain in a structure called the hypothalamus. If you slide your tongue back as far as possible along the roof of your mouth, it will be just a few centimeters below your hypothalamus. The hypothalamus is a part of many regulatory systems, so we will refer to it again in the chapters to come. If the hypothalamus of a mammal's brain is damaged, the animal loses its ability to regulate its body temperature, which then rises in warm environments and falls in cold ones.

The vertebrate thermostat uses feedback information

What information does the vertebrate thermostat use? In many species, the temperature of the hypothalamus itself is the major source of feedback information to the thermostat. Cooling the hypothalamus causes fish and reptiles to seek a warmer environment, and heating the hypothalamus causes them to seek a cooler environment. In mammals, cooling the hypothalamus can stimulate constriction of the blood vessels supplying the skin and increase metabolic heat production. Because it activates these thermoregulatory responses, cooling the hypothalamus causes the body temperature to rise. Conversely, warming the hypothalamus stimulates dilation of blood vessels supplying the skin and sweating or panting, and the overall body temperature falls (Figure 40.18).

708 CHAPTER FORTY

EXPERIMENT

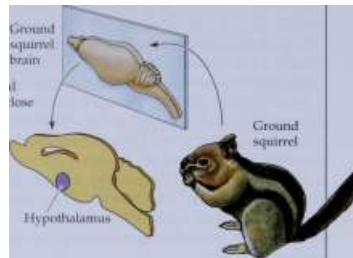
Question: Does the hypothalamus act as a thermostat?

METHOD Heat and cool hypothalamus and measure metabolic heat production.

Ground squirrel

brain

Longitudinal section cut close to midline



Hypothalamus

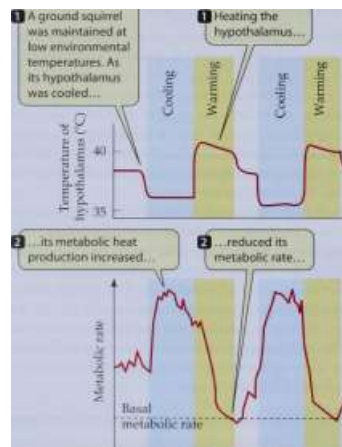
Experiment 1

Q A ground squirrel was maintained at low environmental temperatures. As its hypothalamus was cooled...

f

Experiment 2

Heating the hypothalamus..

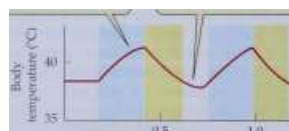


t

...and the animal's body temperature rose.

t

...and the animal's body temperature fell.



0.5 Time (hours)

>nclusion: The ground squirrel's hypothalamus acts as a thermostat.

The hypothalamus appears to generate a set point like a setting on the thermostat of a house. When the temperature of the hypothalamus exceeds or drops below that set point, thermoregulatory responses (the controlled system) are activated to reverse the direction of temperature change. Hence, hypothalamic temperature is a negative feedback signal.

Heating and cooling the hypothalamus show that an animal has separate set points for activating different thermoregulatory responses. If the hypothalamus of a mammal is cooled, the vessels supplying blood to the skin constrict at a specific hypothalamic temperature. A slightly lower hypothalamic temperature initiates shivering. If the hypothalamic temperature is then raised, shivering ceases; then blood vessels supplying the skin dilate; and at still higher hypothalamic temperatures, panting starts. We can describe the characteristics of hypothalamic control of each thermoregulatory response. For example, if we measure metabolic heat production while heating and cooling the hypothalamus (see Figure 40.18), we can describe the results graphically (Figure 40.19). Within a certain range of hypothalamic temperatures, metabolic heat production remains

low and constant, but cooling the hypothalamus below a certain level—a set point—stimulates increased metabolic heat production. The increase in heat production is proportional to how much the hypothalamus is cooled below the set point. This regulatory system is much more sophisticated than a simple on-off thermostat like the one in a house.

The vertebrate thermoregulatory system integrates other sources of information in addition to hypothalamic temperature. It uses information about the temperature of the environment as registered by temperature sensors in the skin. Changes in environmental temperature shift the hypothalamic set points for thermoregulatory responses. As Figure 40.19 shows, in a warm environment you might have to cool the hypothalamus of a mammal to stimulate it to shiver, but in a cold environment you would have to warm the hypothalamus of the same animal to stop it from shivering. The set point for the metabolic heat production response is higher when the skin is cold and lower when the skin is warm.

The temperature of the skin can be considered feedforward information that adjusts the hypothalamic set point. Many other factors also shift hypothalamic set points for responses. Set points are higher during wakefulness than during sleep, and they are higher during the active part of the daily cycle than during the inactive part, even if the animal is awake at both times.

40.18 The Hypothalamus Regulates Body Temperature

The observation that damage to the hypothalamus disrupts thermoregulation led to the finding that hypothalamus acts as a thermostat in the vertebrate body.

EXPERIMENT

Question: How does the mammalian thermoregulatory system integrate temperature information from the environment?

METHOD

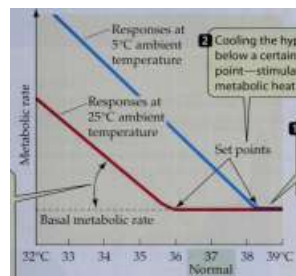
RESULTS

Heat and cool the hypothalamus and measure metabolic rate (see Figure 40.18) at different environmental (ambient) temperatures.

Responses at 5°C ambient temperature

Q Cooling the hypothalamus below a certain level—a set point—stimulates increased metabolic heat production.

Ej The increase in heat production is proportional to how much the hypothalamus is cooled below the set point.



Within a certain range of hypothalamic temperatures, metabolic heat production remains low and constant.

37 Normal

Temperature of hypothalamus

Conclusion: Mammals have different set points for the metabolic heat production response to hypothalamic temperature at different environmental temperatures.

Fevers help the body fight infections

A fever is a rise in body temperature in response to substances called pyrogens that are derived from bacteria or viruses that invade the body. We respond to many infectious illnesses by getting a fever. Growing evidence suggests that fevers are adaptive responses that help the body fight disease-causing organisms.

The presence of a pyrogen in the body causes a rise in the hypothalamic set point for the heat production response. As a result, you shiver, put on a sweater, or crawl under a blanket, and your body temperature rises until it matches the new set point. At the higher body temperature you no longer feel cold, and you may not feel hot, but someone touching your forehead will say that you are "burning up." If you take an aspirin, it lowers your set point to normal. Now you feel hot, take off clothes, and even sweat until your elevated body temperature returns to normal.

Why do we take aspirin for fevers and "feeling crummy"? The pyrogens entering the body are attacked by cells of the immune system called macrophages (see Chapter 19). One of the things the macrophages do is to release chemicals called interleukins, which sound the alarm to other cells of the immune system throughout the body and trigger responses that contribute to feeling crummy. The interleukins also raise the hypothalamic set point for metabolic heat production. Among the intracellular signals trig-

40.19 Adjustable Set Points

Mammals have different set points for the metabolic heat production response to hypothalamic temperature at different environmental temperatures. Other factors, such as being asleep or awake, the time of day, or the presence of a fever, can also affect the set point.

regulated by interleukins are prostaglandins. Aspirin is a potent inhibitor of prostaglandin synthesis, thus explaining how this miracle drug reduces fever and makes us feel better.

Evidence suggests that moderate fevers help the body fight an infection. Some interesting studies were done on lizards that were given access to a heat lamp. These animals kept their body temperatures at about 38°C by adjusting their position with respect to the lamp. After they were injected with disease-causing bacteria, they spent more time close to the lamp and raised their body temperatures to 40°C and higher—they gave themselves fevers. To find out if the fever helped the lizards fight the bacteria, groups of lizards were given equal inoculations of bacteria, but were then placed in different incubators at 34°, 36°, 38°, 40°, and 42°C, respectively. All of the lizards at 34°C and 36°C died, about 25 percent at 38°C survived, and about 75 percent at 40°C and 42°C survived. Apparently fever helped the lizards fight the disease organisms.

However, extreme fevers (for example, 40°C) can be dangerous to humans and must be reduced. Even more modest fevers can be dangerous to people who have weakened hearts or those who are seriously ill. A fetus can be endangered when a pregnant woman has a fever. Fever-re-

ducing drugs may be important in such cases.

Animals can save energy by turning down the thermostat

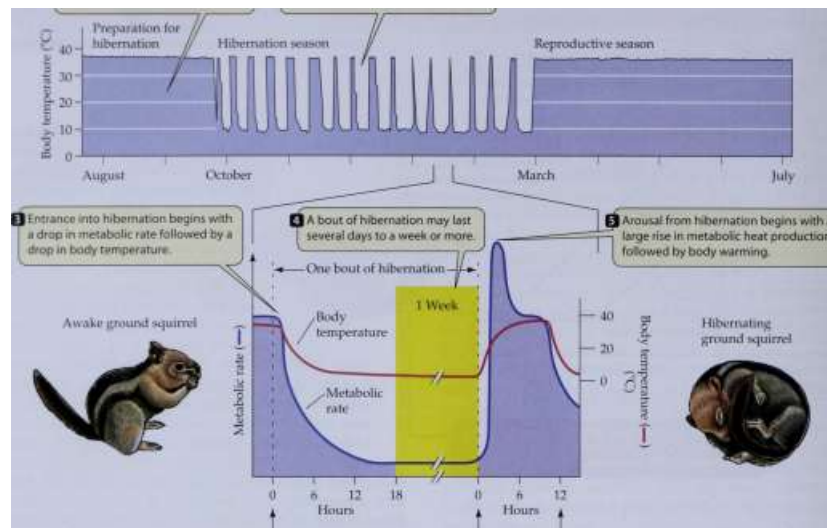
Hypothermia is the condition in which body temperature is below normal. It can result from a natural turning down of the thermostat, or from traumatic events such as starvation (lack of metabolic fuel), exposure, serious illness, or treatment by anesthesia. Many species of birds and mammals use regulated hypothermia as a means of surviving periods of cold and food scarcity. Some become hypothermic on a daily basis. Hummingbirds, for example, are very small endotherms and have a high metabolic rate. They could exhaust their metabolic reserves just getting through a single day without food. Hummingbirds and other small endotherms can extend the period over which they can survive without food by dropping their body temperature during the portion of day when they would normally be inactive. This adaptive hypothermia is called daily torpor. Body temperature can drop 10°-20°C during daily torpor, resulting in an enormous saving of metabolic energy.

710 CHAPTER FORTY

I During more than half the year, a ground squirrel regulates its body temperature near 37°C.

Q During

I winter months, bouts of hibernation are interrupted by brief returns to normal body temperature.



Onset of hibernation

40.20 A Ground Squirrel Enters Repeated Bouts of Hibernation during Winter

At the beginning of each bout of hibernation, the ground squirrel's metabolic rate and body temperature fall. Its body temperature may come into equilibrium with the temperature of its nest and stay at that level for days. The bout is ended by a rise in metabolic heat production that returns body temperature to a normal level.

Regulated hypothermia can also last for days or even weeks, with drops to very low temperatures; this phenomenon is called hibernation (Figure 40.20). During the deep sleep of hibernation, the body's thermostat is turned down to an extremely low level to maximize energy conservation. Arousal from hibernation occurs when the hypothalamic set point returns to a normal level.

Many hibernators maintain body temperatures close to the freezing point during hibernation. The metabolic rate needed to sustain an animal in hibernation may be only one-fiftieth its basal metabolic rate, an enormous saving of metabolic energy. Many species of mammals, such as bats, bears, and ground squirrels, hibernate, but only one species of bird, the poorwill, has been shown to hibernate. The ability of hibernators to reduce their thermoregulatory set points so dramatically probably evolved as an extension of

Onset of arousal

Reentry

the set point decrease that accompanies sleep even in non-hibernating species of mammals and birds.

Chapter Summary

Homeostasis: Maintaining the Internal Environment

► The internal environment consists of the extracellular fluids. Organs and organ systems have specialized functions to keep certain aspects of the internal environment in a constant state. Review Figure 40.1

► Homeostasis is the maintenance of constancy in the internal environment. Homeostasis depends on the ability to control and regulate the functions of organs and organ systems.

Tissues, Organs, and Organ Systems

► Cells that have a similar structure and function make up a tissue. There are four general types of tissues: epithelial, connective, muscle, and nervous. Review Figure 40.2

► Epithelial tissues are sheets of tightly connected cells that cover the body surfaces and line hollow organs. Review Figure 40.3

► Connective tissues support and reinforce other tissues. They generally consist of dispersed cells in an extracellular matrix. Examples are cartilage, bone, blood, and adipose tissue. Review Figure 40.4

► Muscle tissues contract. There are three types: skeletal, cardiac, and smooth.

PHYSIOLOGY, HOMEOSTASIS, AND TEMPERATURE REGULATION 71 1

► There are two types of cells in nervous tissues: Neurons generate and transmit electrochemical signals, and glial cells provide supporting functions for neurons.

► Organs consist of multiple tissue types, and organs make up organ systems. Review Table 40.1

Physiological Regulation and Homeostasis

► Regulatory systems have set points and respond to feedback information. Negative feedback corrects deviations from the set point, positive feedback amplifies responses, and feedforward information changes the set point. Review Figure 40.5

Temperature and Life

► Living systems require a range of temperatures between the freezing point of water and the temperatures that denature proteins.

► Most biological processes and reactions are temperature-sensitive. Q_{10} is a measure of temperature sensitivity. Review Figure 40.6

► Animals that cannot avoid seasonal changes in body temperature have biochemical adaptations that compensate for those changes. These adaptations enable animals to acclimatize to seasonal changes. Review Figure 40.7

Maintaining Optimal Body Temperature

► Homeotherms maintain a fairly constant body temperature most of the time; poikilotherms do not. Endotherms produce metabolic heat to elevate body temperature; ectotherms depend mostly on environmental sources of heat. Review Figure 40.8

► Ectotherms and endotherms can regulate body temperature through behavior. Review Figure 40.9

► Heat exchange between a body and the environment is via radiation, conduction, convection, and evaporation. Review Figure 40.11

► Ectotherms and endotherms can control heat exchange with the environment by altering blood flow to the skin. Review Figure 40.12

► Some ectotherms, such as bees, nocturnal moths, and beetles, can produce metabolic heat to raise their body temperatures. Review Figure 40.13

► Some fish have circulatory systems that function as counter-current heat exchangers to conserve heat produced by muscle metabolism. Review Figure 40.14

Thermoregulation in Endotherms

► Endotherms have high basal metabolic rates. Over a range of environmental temperatures called the thermoneutral zone, the metabolic rate of resting endotherms remains at basal levels. Review Figure 40.15

► When the environmental temperature falls below a lower critical temperature, endotherms maintain their body temperatures through shivering and nonshivering metabolic heat production. Review Figure 40.16

► When the environmental temperature rises above an upper critical temperature, metabolic rate increases as a consequence of active evaporative water loss through sweating or panting.

► Endotherms that live in cold climates have adaptations that minimize heat loss, including a reduced surface area-to-volume ratio and increased insulation.

► Endotherms may dissipate excess heat generated by exercise or the environment via evaporation. The water loss involved in this process can be dangerous to endotherms in dry environments.

The Vertebrate Thermostat

► The vertebrate thermostat is located in the hypothalamus. It has set points for activating thermoregulatory responses. Hypothalamic temperature provides negative feedback information.

► Cooling the hypothalamus induces the constriction of blood vessels and increased metabolic heat production. Heating the hypothalamus induces the dilation of blood vessels and active evaporative water loss. Thermoregulatory behaviors are also induced by changes in hypothalamic temperature. Review Figure 40.18

► Changes in set point reflect the integration of information, such as environmental temperature and time of day, that is relevant to the regulation of body temperature. Review Figure 40.19

► Fever, which results from a rise in set point, helps the body fight infections.

► Adaptations in which set points are reduced to conserve energy include daily torpor and hibernation. Review Figure 40.20

For Discussion

1. In some sheets of epithelial tissue, the cells are joined together with dense extracellular proteins that form "tight junctions," which are extremely impermeable (see Chapter 5). In other epithelial sheets the cells are joined by filamentous extracellular proteins that are strong, but not as impermeable. What do you think are the functions of tight junctions, and where would you expect to find them? Where might you expect to find epithelial sheets with the leakier connections?
2. If the major adaptation of endotherms to cold climates is their insulation, how would you compare the cold adaptations of a polar bear and a seal?
3. Why is an environment above its upper critical temperature more dangerous to an endotherm than an environment below its lower critical temperature?
4. We discussed the vertebrate thermostat by describing experiments done on mammals. Lizards also have a temperature-sensitive hypothalamus. How would you design an experiment on a lizard to see if the temperature of its hypothalamus was important feedback information for its thermoregulation? How would you modify your experiment to see if the lizard also used information from temperature sensors in its skin?
5. If the hypothalamic temperature of a mammal is the feedback information for its thermostat, why does the hypothalamic temperature scarcely change when that animal moves between environments hot enough and cold enough to stimulate the animal to pant and to shiver, respectively?

41

Animal Hormones



^^ In shallow pools around the edge of Lake Tanganyika in east central Africa, brightly colored male cichlid fish stake out territories and vigorously defend them against neighboring males. These dominant males constantly patrol their territories and display their colorful sexual adornments for the benefit of females, who assemble in groups at the edge of the cichlid colony. The females are hard to see because they are inactive and protectively colored. When a female is ready to spawn, and is impressed by a male's territory and display, she enters his territory and lays her eggs in a spawning pit that the male has prepared. The male then fertilizes her

eggs-

At any one time, only about 10 percent of the males in the colony are displaying and holding territories. All the other males are small, nondescript, and nonaggressive like the females. If a dominant male is removed by a predator, however, the nondescript males fight over the vacated territory. The winner rapidly assumes the appearance and behavior of a dominant male: brightly colored, big, aggressive, and attractive to females.

What accounts for this dramatic change? Russell Fernald and his students at Stanford University have shown that soon after the nondescript male's victory, certain cells in his brain enlarge and secrete a chemical message. This message triggers cells in the pituitary gland, which is outside of the brain, to secrete chemical messages in turn. Although secreted in tiny quantities, these molecules enter the blood and are transported around the body. The responses of cells to these chemical messages produce the characteristics of a dominant male.

This change in the male cichlid is one example of how chemical messages, or hormones, can produce and coordinate anatomical, physiological, and behavioral changes in an animal. We explore many other examples in this chapter.

We look first at the nature of hormones and their evolution, and then examine some of their roles in the control of invertebrate life cycles. Most of this chapter is devoted to vertebrate hormones: their functions, control, and molecular mechanisms of action. We pay particular attention to the extensive interactions between the systems of neural and hormonal information. In the process, we discuss several human diseases involving hormonal dysfunction.

Dominant and Non-Dominant Male Cichlids

A dominant male cichlid [*Haplochromis burtoni*] displays bright colors that attract females to his spawning pit.

Hormones and Their Actions

In Chapter 40, we learned that control and regulation require information. This information is transmitted mostly as electric signals and as chemical signals. Electric signals are nerve impulses, a major focus of later chapters on the nervous system. Nerve impulses can be rapidly conducted over long distances to specific targets. Chemical signals are hormones, which are secreted by cells, diffuse locally in the extracellular fluid until they are picked up by the blood, and are distributed by the circulatory system.

Because the secretion, diffusion, and circulation of hormones is much slower than the transmission of nerve impulses, hormones are not useful for controlling rapid actions such as those involved in cichlid fighting. Hormone action is good for coordinating longer-term developmental processes, such as the transition of a nondescript cichlid into a dominant, territorial, breeding male. Hormones control many long-term physiological responses, such as the secretion of digestive enzymes by our guts and the reproductive cycles of many species.

A hormone is a chemical signal produced by certain cells of a multicellular organism and received by cells of the same organism. Cells that secrete hormones are called endocrine cells. To receive the hormonal message, a target cell must have appropriate receptors to which the hormone can bind. The binding of a hormone to its receptor activates mechanisms within the target cell that eventually lead to a response, which may be developmental, physiological, or behavioral. (In the case of the male cichlid, hormone release stimulates all three types of responses.)

Hormonal signaling systems can be distinguished according to the distance over which their messages operate. Some hormones only act on target cells close to their sites of release; others act on target cells at distant locations in the body. Some chemical messages, called pheromones, even exert their effects on other individuals.

Most hormones are distributed in the blood

The classic hormone is a chemical message secreted by cells in minute amounts and distributed throughout the body by the circulatory system (Figure 41.1a). Wherever such a hormone encounters a cell with a receptor to which it can bind, it triggers a response. The nature of the response depends on the responding cell. The same hormone can cause different responses in different types of cells.

Consider the hormone epinephrine. If you step off a curb without looking and a car screeches to a halt right next to you, you jump, your heart starts to thump, and a whole set of protective actions are set in motion. The jump and the initial heart thumping are driven by your nervous system, which can react very quickly. Simultaneously, the nervous system stimulates endocrine cells just above your kidneys (adrenal cells) to secrete epinephrine. Within seconds, epinephrine is diffusing into your blood and circulating around your body to activate the many components of the fight-or-flight response.

Epinephrine acts on the heart, blood vessels, liver, and fat cells. When it binds to its receptors in the heart, it causes the heart to beat faster and more strongly. It binds to receptors in the vessels that supply blood to your digestive tract, causing those vessels to constrict (digestion can wait!). Your heart is pumping more blood, and a greater percentage of that blood is going to the muscles needed for your escape. In the liver, epinephrine stimulates the breakdown of glycogen into glucose for a quick energy supply. In fatty tissue, it stimulates the breakdown of fats as another source of energy. These are just some of the many actions triggered by one hormone. They all contribute to increasing your chances of escaping a dangerous situation.

41.1 Chemical Signaling Systems

(a) The classic hormone is a secreted chemical message that is distributed throughout the body by the circulatory system. (b) An autocrine hormone influences the cell that releases it; paracrine hormones influence nearby cells, (c) Neurotransmitters (see Chapter 44) can be considered paracrine hormones.

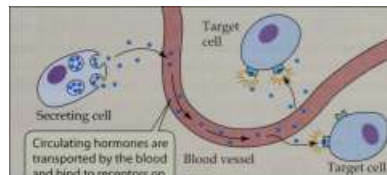
Whether or not a cell responds to a hormone depends on whether it has receptors for that hormone, and how it responds depends on what kind of a cell it is. A single hormone can stimulate many different responses.

Some hormones act locally

Some hormones are released into the extracellular fluids in such tiny quantities, or they are so rapidly inactivated by degradative enzymes, or they are taken up so efficiently by local cells, that the circulation never has the chance to distribute them to distant target cells. Thus, these hormones act only locally. When a hormone affects cells near the secreting cell, it is said to have paracrine function (Figure 41.1b).

An example of a paracrine hormone is histamine, one of the mediators of inflammation. Histamine is released in damaged tissues by specialized cells called mast cells (see Chapter 19). When the skin is cut, the area around the cut becomes inflamed—red, hot, and swollen. Histamine causes this response by dilating the local blood vessels and making them more permeable ("leaky"), which allows

(a) Circulating hormone



Secreting cell

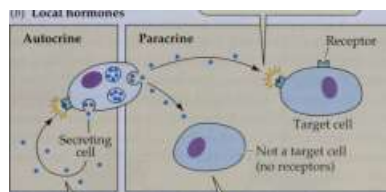
Circulating hormones are transported by the blood and bind to receptors on distant cells.

(b) Local hormones

Blood vessel

Target cell

Paracrine hormones bind to receptors on nearby cells.



^ — ^ Target cell

(9 f Not a target cell

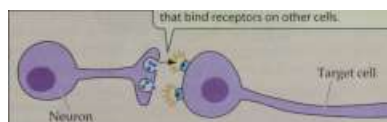
^- - ^ (no receptors)

Autocrine hormones bind to receptors on the cells that secrete them.

Cells without receptors do not respond to a particular hormone.

(c) Neurotransmitter

Neurons secrete chemical neurotransmitters that bind receptors on other cells.



714 CHAPTER FORTY-ONE

blood plasma, including protective blood proteins and white blood cells, to move into the damaged tissue.

A major class of paracrine hormones consists of the various growth factors, which stimulate the growth and differentiation of cells. Growth factors were first discovered when scientists attempted to culture cells outside of the body- Even when given all sorts of nutrients and optimal conditions, the cells did not grow well unless blood plasma or a tissue extract was added to the medium. The components necessary for growth were found to be specific molecules present in very small quantities. At present, about 50 specific growth factors are known, along with a complex group of receptors. Some examples are:

► Nerve growth factor (NGF), which promotes the survival and growth of nerve cells.

- Epidermal growth factor (EGF), which stimulates many kinds of cells to divide
- Vascular endothelial growth factor, which stimulates the growth and branching of blood vessels

The nerve cells called neurons can also be considered paracrine cells. As we will see in Chapter 44, a neuron communicates with another cell by means of a chemical message called a neurotransmitter, which travels over a very small distance to the target cell (Figure 41.1c).

In cases in which receptors for the hormone are on the secreting cell itself, the hormone acts as an autocrine message (see Figure 41.1b). Growth factors are examples of local chemical messages that can also act as autocrine messages for the purpose of negative feedback. The autocrine response prevents the secretory cell from secreting too much of the hormone.

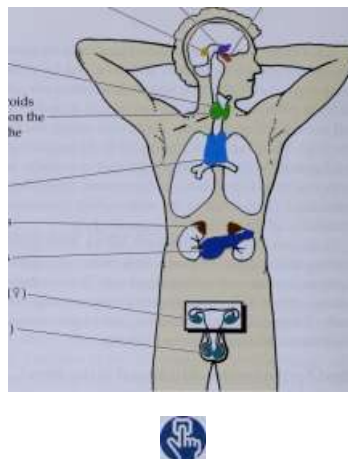
Hormones do not evolve as rapidly as their functions

Chemical signaling between cells exists even in single-celled organisms. Recall the life cycle of slime molds as described in Chapter 27. These protists lead solitary lives and reproduce by mitosis and fission as long as conditions are good. But when food and moisture become scarce, they secrete 3',5'-cyclic adenosine monophosphate (cAMP), which acts as a chemical signal for the individual cells to aggregate into a slug, form a fruiting structure, and release spores. Thus, in this protist, a chemical message passed between cells influences and coordinates behavior, development, and physiology.

The molecule responsible for this very primitive form of chemical communication between slime mold cells— cAMP—is involved in many hormonal signaling systems in multicellular animals. As you learned in Chapter 15, many molecular signals cause the production of cAMP within cells. This "second messenger" mediates a variety of responses within the cell via the phosphorylation of enzymes.

With the evolution of increasingly complex multicellular animals, more and more molecules acquired signaling functions. Also, as physiological systems changed through evolution, many existing molecular signals acquired new functions. As a result, the same chemical substances are used as

Pineal Hypothalamus Pituitary



Thyroid

Parathyroids (located on the back of the thyroid)

Thymus Adrenals Pancreas Ovaries (°) Testes(c?)

47.2 The Endocrine System of Humans

The endocrine system broadcasts chemical signals (hormones) that are received by cells with appropriate receptors. There are nine glands in the human endocrine system, but hormones are also secreted by tissues that are not part of discrete glands.

hormones in widely divergent species, but they may have completely different actions in different species.

The hormone thyroxine, for example, is found in animal species ranging from mollusks and tunicates (sea squirts) to humans. Its function is unknown in invertebrates, but it is produced in an organ that is involved in feeding. In frogs, thyroxine is essential for the metamorphosis from tadpole to adult. In mammals, thyroxine elevates cellular metabolism.

Prolactin is another example of a hormone that evolved different functions in different species. Prolactin stimulates milk production in female mammals after they give birth. In pigeons and doves, prolactin stimulates the production of crop milk, a substance secreted from the crop that is fed to the young. Prolactin causes amphibians to prepare for reproduction by seeking water. In fishes, such as salmon, that migrate between salt water and fresh water to breed, prolactin regulates the mechanisms that maintain osmotic balance with the changing environment. In all of these cases prolactin is involved in reproductive processes, but as those processes have changed through evolution, so has the information signaled by the hormone.

In summary, the structures of the molecules involved in chemical signaling are highly conserved—they have changed little throughout evolution—but their functions have changed dramatically.

Endocrine glands secrete hormones

Some endocrine cells are distributed as single cells within a tissue. Many hormones of the digestive tract, for example, are produced and secreted by isolated cells in the lining of the tract. As the contents of the digestive tract come into contact with these cells, they release their hormones, which

enter the blood and, like epinephrine, circulate throughout the body and activate cells that have appropriate receptors. Many hormones, however, are secreted by aggregations of endocrine cells that form secretory organs called endocrine glands.

Animals have two types of glands. Exocrine glands, such as sweat glands and salivary glands, release secretions that are not hormones through ducts that lead outside the body. Sweat gland ducts, for example, open onto the surface of the skin, and salivary gland ducts open into the mouth. Glands that secrete hormones and do not have ducts are called endocrine glands; they secrete their products directly into the extracellular fluid. Vertebrates have nine discrete endocrine glands, which collectively make up the endocrine system (Figure 41.2).

Hormonal Control of Molting and Development in Insects

Many hormones of invertebrate animals have multiple functions. In this chapter we cannot do justice to the diversity of hormones in the invertebrates, but we'll discuss two important aspects of the lives of many invertebrates that are controlled by hormonal mechanisms: molting and metamorphosis.

Hormones from the head control molting in insects

Because insects have rigid exoskeletons, their growth is episodic, punctuated with molts (shedding) of the exoskeleton (see Chapter 32). Each growth stage between two molts is called an instar. The British physiologist Sir Vincent Wigglesworth was a pioneer in the study of the hormonal control of growth and development in insects.

Wigglesworth conducted experiments on the bloodsucking bug *Rhodnius*, which undergoes incomplete metamorphosis. Upon hatching, *Rhodnius* is nearly a miniature version of an adult, but it lacks some adult features. *Rhodnius* molts five times before developing into a mature adult; a blood meal triggers each episode of molting and growth.

Rhodnius is a hardy experimental animal; it can live a long time even after it is decapitated. If decapitated about an hour after it has a blood meal, *Rhodnius* may live for up to a year, but it does not molt. If decapitated a week after its blood meal, it does molt (Figure 41.3, Experiment 1). These observations led Wigglesworth to the hypothesis that something diffusing slowly from the head controls molting.

The proof of this hypothesis came from a clever experiment in which Wigglesworth decapitated two *Rhodnius*: one that had just had its blood meal and another that had had its blood meal a week earlier. The two decapitated bodies

41.3 A Diffusible Substance Triggers Molting

The effect of time since the last blood meal on *Rhodnius* molting led Sir Vincent Wigglesworth to hypothesize that some substance was diffusing slowly through the insect's body. Further experiments showed that molting is indeed controlled by a substance— a hormone—diffusing from the head.

EXPERIMENT

Observation: A blood meal stimulates the bug, *Rhodnius*, to molt. Hypothesis: Molting is controlled by substances diffusing from the head.

Experiment 1

METHOD

Decapitate juvenile bugs at different times after a blood meal.



Juvenile bug (third instar)

/

Decapitation 1

hour after blood meal

Decapitation 1

week after blood meal

RESULTS



I



Does not molt (remains a juvenile)

^

Molts into an adult

_A

Whether a decapitated *Rhodnius* will molt depends on the interval between a blood meal and the decapitation.

Experiment 2

METHOD

Decapitate third instars at different times after a blood meal.



Decapitation 1 Decapitation 2

hour after blood meal week after blood meal

RESULTS



Join bugs with glass tube



Unjoined bug does not molt



Unjoined bug molts into adult



Both bugs molt into adults

Conclusion: A diffusible substance from the head region is necessary for molt.

716 CHAPTER FORTY-ONE

were connected with a short piece of glass tubing—and they both molted (Figure 41.3, Experiment 2). Thus one or more substances from the bug fed a week earlier crossed through the glass tube and stimulated molting in the other bug.

We now know that two hormones working in sequence regulate molting:

- ▶ Cells in the brain produce brain hormone.
- ▶ Brain hormone is transported to and stored in a pair of structures attached to the brain, the corpora cardiaca (singular corpus car-diacum).
- ▶ After appropriate stimulation (which for *Rhodnius* is a blood meal) the corpora cardiaca release brain hormone, which diffuses to an endocrine gland, the prothoracic gland.
- ▶ Brain hormone stimulates the prothoracic gland to release the hormone ecdysone.
- ▶ Ecdysone diffuses to target tissues and stimulates molting.

The control of molting by brain hormone and ecdysone is a general mechanism in insects. The nervous system receives various types of information relevant in determining the optimal timing for growth and development. It makes sense, therefore, that the nervous system should control the endocrine gland that produces the hormone that orchestrates all the physiological processes involved in development and molting. Later in this chapter we will see similar links between the nervous system and endocrine glands in vertebrates.

Juvenile hormone controls development in insects

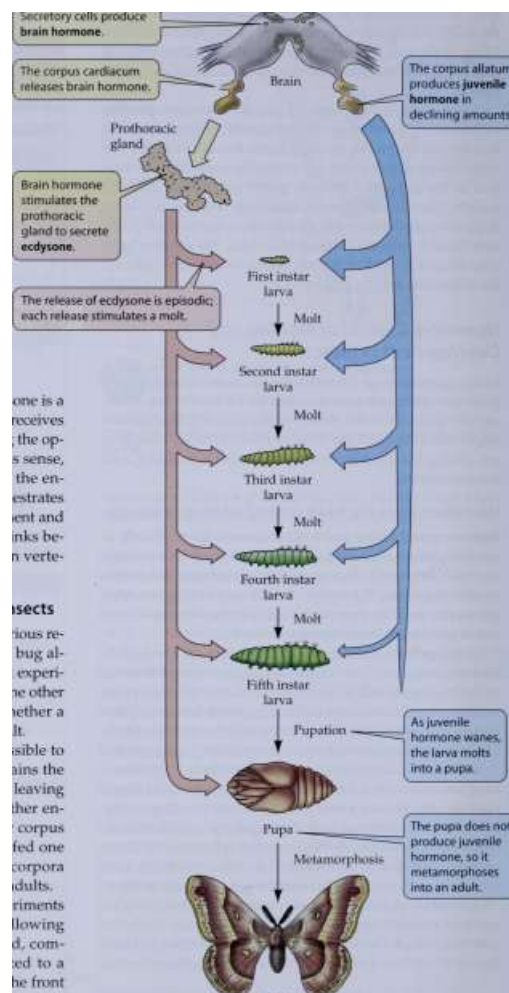
The *Rhodnius* decapitation experiments yielded a curious result: Regardless of the instar used, the decapitated bug always molted directly into an adult form. Additional experiments by Wigglesworth demonstrated that a hormone other than those responsible for molting determines whether a bug molts into another juvenile instar or into an adult.

Because the head of *Rhodnius* is long, it was possible to remove just the front part of the head, which contains the cells that secrete and release brain hormone, while leaving intact the rear part. That rear part contains two other endocrine structures called the corpora allata (singular corpus allatum). When fourth-instar bugs that had been fed one week earlier were partly decapitated, leaving the corpora allata intact, they molted into fifth instars, not into adults.

This experiment, was followed up by more experiments using glass tubes to connect individual bugs, allowing body fluid transfer between them. When an unfed, completely decapitated, fifth-instar bug was connected to a fourth-instar bug that had been fed and had only the front part of its head removed, both bugs molted into juvenile forms. A substance coming from the rear part of the head of the fourth-instar bug prevented the expected result that both bugs would molt into adult forms.

We now know that the substance is juvenile hormone and that it comes from the corpora allata. As long as juvenile

Secretory cells produce brain hormone.



Adult

41.4 Complete Metamorphosis

Butterflies and moths undergo complete metamorphosis, in which the feeding larvae (caterpillars) bear no resemblance to the reproductive adult. Three hormones control molting and metamorphosis in the silkworm moth *Hyalophora cecropia*.



juvenile hormone is present, *Rhodnius* molts into another juvenile instar. The corpora allata normally stop producing juvenile

The control of development by juvenile hormone is more complex in insects that, like butterflies, undergo complete metamorphosis. These animals undergo dramatic developmental changes between instars. The fertilized egg hatches into a larva, which feeds and molts several times, becoming bigger and bigger. Then it enters an inactive stage called a pupa. It undergoes major body reorganization as a pupa, and finally emerges as an adult.

In our perpetual war against insects, juvenile hormone is a new weapon. Synthetic forms of juvenile hormone can be distributed in the environment to prevent the development of juvenile insects into adults capable of reproduction. However, as you might expect from the fact that hormone structures are highly conserved, such a weapon is not without potentially serious side effects. First, it is not selective in its effects on insects and can affect species that are beneficial as well as those that are pests. Second, the synthetic juvenile hormone could have actions in vertebrates that are not yet known.

The diagram shows a sagittal section of the human brain. A red arrow points from the hypothalamus, located at the base of the brain, to the pituitary gland, which is situated just below it. A callout box points to the hypothalamus with the text: "The hypothalamus is the brain's master gland." The label "Hypothalamus" is also present at the bottom of the diagram.

The diagram illustrates the pituitary gland's location and components. The **Hypothalamus** is shown at the top, with a callout stating: "Hypothalamus produce the hormones that control the posterior pituitary". The **Pituitary stalk** connects the hypothalamus to the **Pituitary gland**. The gland is divided into the **Anterior pituitary** (adenohypophysis) and the **Posterior pituitary** (neurohypophysis). **Capillaries** are shown within the stalk. A red vessel labeled "flowing blood" is shown entering the anterior pituitary. A caption at the bottom states: "The hormones secreted by the..."

The list of chemical messages in the bodies of vertebrates is long and growing longer. To make the subject manageable, we focus mostly on the hormones of humans—how they function and how they are controlled. Table 41.1 presents an overview of the hormones of humans (most of which are found in all other mammals as well). Notice that the column listing the target

tissues of these hormones includes every organ system of the body.

We begin this survey with the pituitary gland because it plays a central role in the endocrine system. The pituitary is a link between the nervous system and many endocrine glands. It secretes some hormones that are actually produced by neurons in the brain, and under the influence of still other brain hormones, it produces a number of its own hormones, which control the activities of various endocrine glands throughout the body. For these reasons, the pituitary has been called "the master gland."

The pituitary develops from outpocketings of the mouth and brain

The pituitary gland sits in a depression at the bottom of the skull just over the back of the roof of the mouth (Figure 41.5). It is attached to the part of the brain called the hypothalamus, which is involved in many homeostatic regulatory systems (see Chapter 40).

The pituitary has two distinct parts that have different functions and separate origins during development. The anterior pituitary originates as an outpocketing of the embryonic mouth cavity, and the posterior pituitary originates as an outpocketing of the developing brain in the region that becomes the hypothalamus.

the posterior pituitary. The posterior pituitary releases two hormones, antidiuretic hormone and oxytocin. Both are small peptides synthesized in neurons in the hypothalamus.

718 CHAPTER FORTY-ONE

Hormones that are produced and released by neurons are called neurohormones. Antidiuretic hormone and oxytocin move down long extensions (axons) of the neurons that produce them, through the pituitary stalk into the posterior pituitary, where they are stored in the nerve endings (see Figure 41.5). How do they move down the axons? In the bodies of the neurons, these neurohormones are packaged into vesicles. Proteins called kinesins grab onto the vesicles and, powered by ATP, "walk" step by step down microtubules in the axons (see Figure 4.25).

The main action of antidiuretic hormone (ADH) is to increase the amount of water conserved by the kidneys. When ADH secretion is high, the kidneys resorb more water and produce only a small volume of highly concentrated urine. When ADH secretion is low, the kidneys produce a large volume of dilute urine.

The posterior pituitary increases its release of ADH whenever blood pressure falls or the blood becomes too salty. We will discuss the mechanism of ADH action in Chapter 51. ADH is also known as vasopressin because it

ANIMAL HORMONES 719

nri . 1 Principal Hormones of Humans (continued)

SECRETING TISSUE OR GLAND

HORMONE

CHEMICAL NATURE

TARGET(S)

IMPORTANT PROPERTIES OR ACTIONS

Adrenal medulla

Adrenal cortex

Epinephrine, norepinephrine

Glucocorticoids (Cortisol)

Modified amino acids

Steroids

Mineralocorticoids Steroids (aldosterone)

Heart, blood

vessels, liver,

fat cells Muscles,

immune

system, other

tissues Kidneys

Stimulate fight-or-flight reactions:

increase heart rate, redistribute blood to muscles, raise blood sugar

Mediate response to stress; reduce metabolism of glucose, increase metabolism of proteins and fats; reduce inflammation and immune responses

Stimulate excretion of potassium ions and reabsorption of sodium ions

Promotes digestion of food by stimulating release of digestive juices; stimulates stomach movements that mix food and digestive juices

Stimulate secretion of bicarbonate solution by ducts of pancreas

Stimulates secretion of digestive enzymes by pancreas and other digestive juices from liver; stimulates contractions of gallbladder and ducts

Inhibits digestive activities in the stomach Involved in biological rhythms

Stimulate development and maintenance of female characteristics and sexual behavior

Sustains pregnancy; helps maintain secondary female sexual characteristics

Stimulate development and maintenance of male sexual behavior and secondary male sexual characteristics; stimulate sperm production

Have many diverse actions Increases sodium ion excretion

also causes the constriction of peripheral blood vessels as a means of elevating blood pressure.

When a woman is about to give birth, her posterior pituitary releases oxytocin, which stimulates the contractions of the muscles that push the baby out of her body. Oxytocin also brings about the flow of milk from the mother's breasts. The baby's suckling stimulates nerve cells in the mother, causing the secretion of oxytocin. Even the sight and sounds of her baby can cause a nursing mother to secrete oxytocin and release milk from her breasts.

the anterior pituitary. Four hormones released by the anterior pituitary (thyrotropin, adrenocorticotropin, luteinizing hormone, and follicle-stimulating hormone) control the activities of other endocrine glands and thus are called tropic hormones (see Figure 41.7). Each tropic hormone is produced by a different type of pituitary cell. We will say more about these tropic hormones when we describe their target

glands (thyroid, adrenal cortex, testes, and ovaries) later in this chapter and in the next.

The other hormones produced by the anterior pituitary influence tissues that are not endocrine glands. These hormones are growth hormone, prolactin, melanocyte-stimulating hormone, endorphins, and enkephalins.

Growth hormone (GH) consists of about 200 amino acids and acts on a wide variety of tissues to promote growth directly and indirectly. One of its important direct effects is to stimulate cells to take up amino acids. Growth hormone promotes growth indirectly by stimulating the liver to produce chemical messages that stimulate the growth of bone and cartilage. Thus, in some of its actions, growth hormone can also be considered a tropic hormone.

Overproduction of growth hormone in children causes gigantism, and underproduction causes dwarfism (Figure 41.6). Beginning in the late 1950s, children diagnosed as having a serious deficiency of growth hormone were treated



47.6 Effects of Abnormal Amounts of Growth Hormone

(a) Overproduction of growth hormone in childhood causes gigantism. This photo from 1939 shows a young man who is more than 8 feet tall standing next to his father, who is just under 6 feet tall, (b) Underproduction of growth hormone during childhood results in pituitary dwarfism. The man on the left is P.T. Barnum, the circus entrepreneur. The man on the right is Charles Stratton, a dwarf, who appeared in Barnum's circus under the name General Tom Thumb.



with human growth hormone extracted from human pituitaries in cadavers. The treatment was successful in stimulating substantial growth, but it could be made available to only small numbers of patients. A year's supply of human growth hormone for one individual required up to 50 pituitaries. In the mid-1980s, scientists using genetic engineering technology isolated the gene for human growth hormone and introduced it into bacteria, which produced enough of the hormone to make it widely available.

Preventing pituitary dwarfism is now feasible and affordable, but the availability of growth hormone raises new questions. Should every child at the lower end of the height charts be treated? Should a normal child whose parents think basketball stardom is assured if she is tall be given growth hormone? These types of questions are impossible to answer with scientific data alone.

Prolactin, another hormone produced by the anterior pituitary, stimulates the production and secretion of milk in female mammals. In some mammals, prolactin also functions as an important hormone during pregnancy. In human

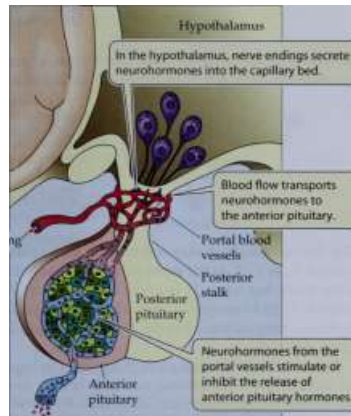
males, prolactin plays a role along with other pituitary hormones in controlling the endocrine function of the testes.

Endorphins and enkephalins are the body's "natural opiates." In the brain, these molecules act as neurotransmitters in pathways that control pain. The significance of their release from the anterior pituitary is unknown. Interestingly, the production of endorphins and enkephalins in the pituitary is encoded by the same gene that encodes at least two other pituitary hormones. The gene actually encodes a large parent molecule called pro-opiomelanocortin. This large protein molecule is cleaved to produce several peptides, some of which have hormonal functions. Adrenocorticotropic hormone, melanocyte-stimulating hormone, endorphins, and enkephalins all result from the cleavage of pro-opiomelanocortin.

THE ANTERIOR PITUITARY IS CONTROLLED BY HYPOTHALAMIC

neurohormones Because the anterior pituitary produces tropic hormones that control other endocrine tissues, it acquired the designation "master gland." But we now

Inflowing blood I



Anterior pituitary

Neurohormones from the portal vessels stimulate or inhibit the release of anterior pituitary hormones.

• 0



Anterior pituitary hormones leave gland in the blood.

47.7 Hormones from the Hypothalamus Control the Anterior Pituitary

Neurohormones produced in tiny quantities by cells in the hypothalamus are transported to the anterior pituitary through a system of portal blood vessels. These releasing and release-inhibiting hormones control the activities of anterior pituitary endocrine cells.

know that this "master" is under still higher control by the hypothalamus, and that their interaction integrates nervous system and endocrine system functions.

The hypothalamus receives information about conditions in the body and in the external environment through the nervous system. If the connection between the hypothalamus and the pituitary is experimentally cut, pituitary hormones are no longer released in response to changes in the environment or in the body. If pituitary cells are maintained in culture, extracts of hypothalamic tissue stimulate

ANIMAL HORMONES 721

some of those cells to release their hormones into the culture medium. Therefore, scientists hypothesized that secretions of the hypothalamic cells control the activities of anterior pituitary cells.

Although hypothalamic neurons do not extend into the anterior pituitary as they do into the posterior pituitary, a special set of portal blood vessels connects the hypothalamus and the anterior pituitary (Figure 41.7). It was thus proposed that secretions from nerve endings in the hypothalamus enter the blood and are conducted down the portal vessels to the anterior pituitary, where they cause the release of anterior pituitary hormones.

In the 1960s, two large teams of scientists, led by Roger Guillemin and Andrew Schally, initiated the search for the hypothalamic neurohormones. Because the amounts of such hormones in any individual mammal would be tiny, massive numbers of hypothalami from pigs and sheep were collected from slaughterhouses and shipped to laboratories in refrigerated trucks. One extraction effort began with the hypothalami from 270,000 sheep and yielded only 1 mg of purified thyrotropin-releasing hormone, or TRH, which was the first hypothalamic releasing (that is, release-stimulating) hormone isolated and characterized. Biochemical analysis of this pure sample revealed that TRH is a simple tripeptide consisting of glutamine, histidine, and proline. TRH causes certain anterior pituitary cells to release the tropic hormone thyrotropin, which in turn stimulates the activity of the thyroid gland.

Soon after discovering thyrotropin-releasing hormone, Guillemin's and Schally's teams identified gonadotropin-releasing hormone, which stimulates certain anterior pituitary cells to release the tropic hormones that control the activity of the gonads (the ovaries and the testes). For these discoveries, Guillemin and Schally received the 1972 Nobel prize in medicine. Many more hypothalamic neurohormones, including both releasing hormones and release-inhibiting hormones, are now known (Table 41.2).

Negative feedback loops control hormone secretion

As well as being controlled by hypothalamic releasing and release-inhibiting hormones, the endocrine cells of the ante-

Releasing and Release-Inhibiting Neurohormones of the Hypothalamus

NEUROHORMONE

ACTION

Thyrotropin-releasing hormone (TRH) Gonadotropin-releasing hormone (GnRH)

Prolactin release-inhibiting hormone

Prolactin-releasing hormone

Somatostatin (growth hormone release-inhibiting hormone)

Growth hormone-releasing hormone Adrenocorticotropin-releasing hormone Melanocyte-stimulating hormone release-inhibiting hormone

Stimulates thyrotropin release

Stimulates release of follicle-stimulating hormone and luteinizing hormone

Inhibits prolactin release

Stimulates prolactin release

Inhibits growth hormone release; interferes with thyrotropin

release Stimulates growth hormone release Stimulates adrenocorticotropin release Inhibits release of melanocyte-stimulating hormone

722 CHAPTER FORTY-ONE

External conditions

©

or

o

—©-► = Stimulates —Q+ = Inhibits

r G

Long-loop

negative

feedback

-Q+



Hypothalamus



I

Releasing hormone



Short-loop

negative

feedback

Tropic hormone

©



Endocrine gland



i

Hormone

47.8 Multiple Feedback Loops Control Hormone Secretion

Multiple feedback loops regulate the chain of command from hypothalamus to anterior pituitary to endocrine glands.

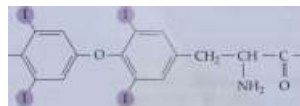
rior pituitary are also under negative feedback control by the hormones of the target glands they stimulate (Figure 41.8). For example, the hormone Cortisol, produced by the adrenal gland in response to adrenocorticotropin, returns to the pituitary in the circulating blood and inhibits further adrenocorticotropin release. Cortisol also acts as a negative feedback signal at the level of the hypothalamus, inhibiting the release of adrenocorticotropin-releasing hormone. In some cases a tropic hormone of the anterior pituitary also exerts negative feedback control on the hypothalamic cells producing the corresponding releasing hormone.

Thyroxine controls cell metabolism

The thyroid gland wraps around the front of the windpipe (trachea) and expands into a lobe on either side (see Figure 41.2). The thyroid gland produces the hormones thyroxine and calcitonin.

Thyroxine is synthesized in thyroid cells from two molecules of diiodotyrosine, which is the amino acid tyrosine with two atoms of iodine chemically bonded to it. Thus, a thyroxine molecule has four atoms of iodine, and is called

HO



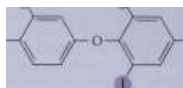
OH

Thyroxine (T₄)

Thyroid cells also produce triiodothyronine, a version of thyroxine that has only three atoms of iodine and is called

II

HO



CH₂—CH—C—OH

NH₂ O

Triiodothyronine (T₃)

The thyroid usually makes and releases about four times as much T₄ as T₃. T₃ is the more active hormone in the cells of the body, but when T₄ is in circulation, it can be converted to T₃ by an enzyme. Therefore, when you read about thyroxine, keep in mind that the actions discussed are primarily due to T₃.

Thyroxine in mammals plays many roles in regulating cell metabolism. It elevates the metabolic rates of most cells and tissues and promotes the use of carbohydrates rather than fats for fuel. Exposure to cold for several days leads to an increased release of thyroxine, an increased conversion of T₄ to T₃, and an increase in basal metabolic rate. Thyroxine is especially crucial during development and growth, as it promotes amino acid uptake and protein synthesis by cells. Insufficient thyroxine in a human fetus or growing child greatly retards physical and mental growth, resulting in a condition known as cretinism.

The tropic hormone thyrotropin from the anterior pituitary activates the thyroid cells that produce thyroxine (Figure 41.9). TRH (thyrotropin-releasing hormone) produced in the hypothalamus and transported to the anterior pituitary through the portal blood vessels activates the thy-rotropin-producing pituitary cells. The brain uses environmental information such as temperature or day length to determine whether to increase or decrease the secretion of TRH. There is a very important negative feedback loop in this sequence of steps: Circulating thyroxine inhibits the response of the pituitary cells to TRH. Less thyrotropin is released when thyroxine levels are high, and more thyrotropin is released when thyroxine levels are low.

Thyroid dysfunction causes goiter

A goiter is an enlarged thyroid gland, which causes a pronounced bulge on the front and sides of the neck. Goiter can be associated with either hyperthyroidism (very high levels of thyroxine) or hypothyroidism (very low levels of thyroxine). The control diagram in Figure 41.9 helps explain how two very different conditions can result in the same symptom.

Hyperthyroid goiter results when the negative feedback mechanism fails to turn off the thyroid cells even though blood levels of thyroxine are high. The most common cause of hyperthyroidism is an autoimmune disease in which an antibody to the thyrotropin receptor is produced. This antibody can bind to the receptor and cause the thyroid cells to produce and release thyroxine. Even though blood levels of thyrotropin may be quite low because of the negative feed-

Cold exposure

©

—©—► = Stimulates - o+ = Inhibits

ANIMAL HORMONES 723

Hyp

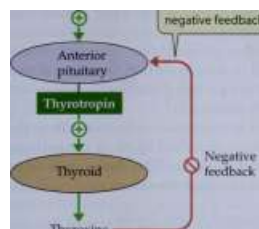
Hypothalamus

Thy

7

Thyrotropin-releasing hormone

High thyroxine levels act as negative feedback.



^ Negative ^ feedback

Thyroxine

47.9 Regulation of Thyroid Function in Response to Cold

Exposure to cold temperatures stimulates the hypothalamus to produce thyrotropin-releasing hormone, which stimulates anterior pituitary cells to secrete thyrotropin, which in turn stimulates the thyroid to release thyroxine.

back from thyroxine, the thyroid remains maximally stimulated, and it grows bigger. Hyperthyroid patients have high metabolic rates, are jumpy and nervous, usually feel hot, and may have a buildup of fat behind the eyeballs, causing their eyes to bulge.

Hypothyroid goiter results when there is not enough circulating thyroxine to turn off thyrotropin production. Its most common cause is a deficiency of dietary iodide, without which the thyroid gland cannot make thyroxine. Without sufficient thyroxine, thyrotropin levels remain high, so the thyroid continues to produce large amounts of nonfunctional thyroxine and becomes very large. The symptoms of hypothyroidism are low metabolism, intolerance of cold, and general physical and mental sluggishness.

Worldwide, goiter affects about 5 percent of the population. The addition of iodide to table salt has greatly reduced the incidence of the condition in industrialized nations, but goiter is still common in the less industrialized countries of the world.

Calcitonin reduces blood calcium

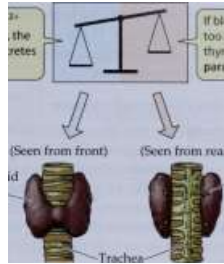
Another hormone released by the thyroid gland is calcitonin, although it is not produced by the same cells that produce thyroxine. Calcitonin helps reduce the levels of calcium circulating in the blood (Figure 41.10).

Bone is a huge repository of calcium in the body and is continually being remodeled. Cells called osteoclasts break down bone and release calcium; osteoblasts, on the other hand, use circulating calcium to deposit new bone. Calcitonin decreases the activity of osteoclasts and stimulates the activity of osteoblasts, thus shifting the

IMBALANCE

If blood Ca^{2+} is too high, the thyroid secretes calcitonin.

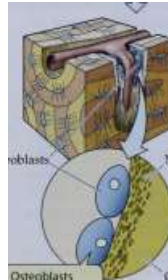
Thyroid gland



If blood Ca^{2+} is too low, the parathyroids secrete parathormone.

Parathyroid gland

Calcitonin stimulates osteoblasts to take up Ca^{2+} from blood and lay down new bone.



Parathormone stimulates osteoclasts to resorb bone and return Ca^{2+} to blood; it also stimulates the absorption of calcium from the intestines and decreased loss of calcium from the kidneys.

Osteoblasts

New bone

Osteoblasts build new bone using calcium from the blood.

Older bone



Bone Blood vessel

Osteoclasts

Blood Ca^{2+} level falls.



Osteoclasts breakdown bone and release calcium.

Blood Ca^{2+} level rises.



Calcium homeostasis 9-11 mg/ 100 ml blood

HOMEOSTASIS

47.70 Hormonal Regulation of Calcium

Calcitonin and parathormone help regulate blood calcium levels. Bone can be a source (site of production) or a sink (site of utilization or storage) for calcium.

724 CHAPTER FORTY-ONE

balance from adding calcium ions to the blood to removing them.

Calcitonin plays an important role in preventing bone loss in women during pregnancy. However, regulation of blood calcium levels is influenced more strongly by parathormone than it is by calcitonin.

Parathormone elevates blood calcium

The parathyroid glands are four tiny structures embedded on the surface of the thyroid gland. Their single hormone product is parathyroid hormone, or parathormone, a critical control in the regulation of blood calcium levels. Growth and remodeling of bone require calcium, and muscle contraction and nerve function are severely impaired if the blood calcium level rises or falls by as little as 30 percent of normal values.

A decrease in blood calcium triggers the release of parathormone, which stimulates osteoclasts to dissolve bone and release calcium to the blood (see Figure 41.10). Parathormone also prevents calcium in the blood from being lost in the urine by promoting calcium resorption by the kidneys. It also promotes the activation of vitamin D, which stimulates the digestive tract to absorb calcium from food.

Parathormone and calcitonin act antagonistically to regulate blood calcium levels: Parathormone elevates and calcitonin reduces. A similar antagonistic relationship is true of hormones of the pancreas, which regulate blood glucose levels.

Insulin and glucagon regulate blood glucose

Before the 1920s, diabetes mellitus was a fatal disease, characterized by weakness, lethargy, and body wasting. The disease was known to be connected somehow with the pancreas, a gland located just below the stomach, (see Figure 41.2), and with abnormal glucose metabolism, but the link was not clear.

Today we know that diabetes mellitus is caused by a lack of the hormone insulin, or by a lack of responsiveness of target tissues to that hormone. For patients in which the hormone is lacking, insulin replacement therapy is an extremely successful treatment. At present, more than 1.5 million people with diabetes in the United States lead almost normal lives through the use of manufactured insulin.

Insulin binds to a receptor on the plasma membranes of target cells, and this insulin-receptor complex allows glucose to enter the cell (see Figure 15.7). In the absence of insulin or insulin receptors, glucose accumulates in the blood

of glucose. As a result, the body of the untreated diabetic wastes away, and critical tissues and organs are damaged.

For centuries the prospects for a person with diabetes were bleak. A change in this outlook came almost overnight in 1921, when medical doctor Frederick Banting and medical student Charles Best of the University of Toronto discovered that they could reduce the symptoms of diabetes by injecting an extract they prepared from pancreatic tissue. The active component of the extract that Banting and Best prepared was found to be a small protein hormone—insulin—consisting of 51 amino acids.

Insulin is produced in clusters of endocrine cells in the pancreas. These clusters are called islets of Langerhans after the German medical student who discovered them. There are several types of cells in the islets:

- ▶ Beta (β) cells produce and secrete insulin.
- ▶ Alpha (α) cells produce and secrete the hormone glucagon, which has effects opposite those of insulin.
- ▶ Delta (δ) cells produce the hormone somatostatin.

The rest of the pancreas is an exocrine gland that produces enzymes and secretions that travel through ducts to the intestine, where they play roles in digestion.

After a meal, the concentration of glucose in the blood rises as glucose is absorbed from the food in the gut. This increase stimulates the pancreas to release insulin. Insulin stimulates cells to use glucose as fuel and to convert it into storage products such as glycogen and fat. When the gut contains no more food, the glucose concentration in the blood falls, and the pancreas stops releasing insulin. As a result, most cells of the body shift to using glycogen and fat rather than glucose for fuel. If the concentration of glucose in the blood falls below normal, the islet cells release glucagon, which stimulates the liver to convert glycogen back to glucose to resupply the blood. These effects will be discussed in greater detail in Chapter 50.

Somatostatin is a hormone of the brain and the gut

Somatostatin is released from the pancreas in response to rapid rises in glucose and amino acids in the blood. This hormone has paracrine functions within the islets: It inhibits the release of both insulin and glucagon. Its actions outside the pancreas slow the digestive activities of the gut. Pancreatic somatostatin extends the period of time during which nutrients are absorbed from the gut and used by the cells of the body. Somatostatin also acts as a hypothalamic neurohormone that inhibits the release of growth hormone

until it is lost in the urine. High levels of blood glucose and thyrotropin by the pituitary,

cause water to move from cells into the blood by osmosis, and the kidneys increase urine output to excrete excess fluid volume from the blood.*

Glucose uptake by most cells is impaired without insulin, so those cells must use fat and protein for fuel instead

The name diabetes refers to the copious production of urine. Mellitus (Greek for "honey") reflects the fact that the urine of an untreated diabetic is sweet.

The adrenal gland is two glands in one

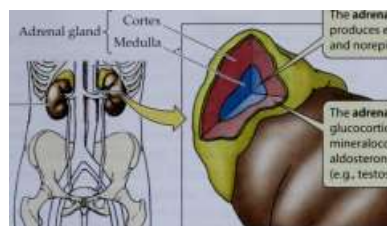
An adrenal gland sits above each kidney just below the middle of your back. Functionally and anatomically, an adrenal gland consists of a gland within a gland (Figure 41.11). The core, called the adrenal medulla, produces the hormone epinephrine (also known as adrenaline) and, to a lesser degree, norepinephrine (or noradrenaline). Surrounding the medulla (as an apricot surrounds its pit) is the adrenal cortex.

Adrenal gland

The adrenal medulla

produces epinephrine and norepinephrine.

Kidney



adrenal cortex produces glucocorticoids (e.g., Cortisol), mineralocorticoids (e.g., aldosterone), and sex steroids e.g., testosterone).

41.11 The Adrenal Gland Has an Outer and an Inner Portion

An adrenal gland, consisting of an outer cortex and an inner medulla, sits on top of each kidney. The medulla and the cortex produce different hormones.

The adrenal cortex, which produces other hormones. The medulla develops from nervous system tissue and is under the control of the nervous system; the cortex is under hormonal control, largely by adrenocorticotropic (ACTH) from the anterior pituitary.

The adrenal medulla. The adrenal medulla produces epinephrine in response to stressful situations, arousing the body to action. As we saw earlier in this chapter, epinephrine increases heart rate, breathing rate, and blood pressure, and diverts blood flow to active skeletal muscles and away from the gut. These fight-or-flight reactions can be stimulated by physically threatening events, such as encountering a mugger, or by events that are mentally stressful, such as giving a public speech or taking a test. In situations of mental stress, many of the responses (such as increased heart and breathing rates) that would be useful for escaping physical danger are not useful, and can even be inconvenient or harmful.

Epinephrine and norepinephrine (a neurotransmitter involved in physiological regulation) bind to receptors on the surfaces of target cells. These receptors can be grouped into two general types, α -adrenergic and β -adrenergic receptors (each with at least two subtypes), which stimulate different actions within cells. Epinephrine acts equally on both types, but norepinephrine acts mostly on α -adrenergic receptors. Therefore, drugs called β -blockers, which selectively block β -adrenergic receptors, can reduce the fight-or-flight responses to epinephrine without disrupting the regulatory functions of norepinephrine.

The adrenal cortex. The cells of the adrenal cortex use cholesterol to produce three classes of steroid hormones. (Figure 41.12). Collectively, these classes of hormones are called the corticosteroids."

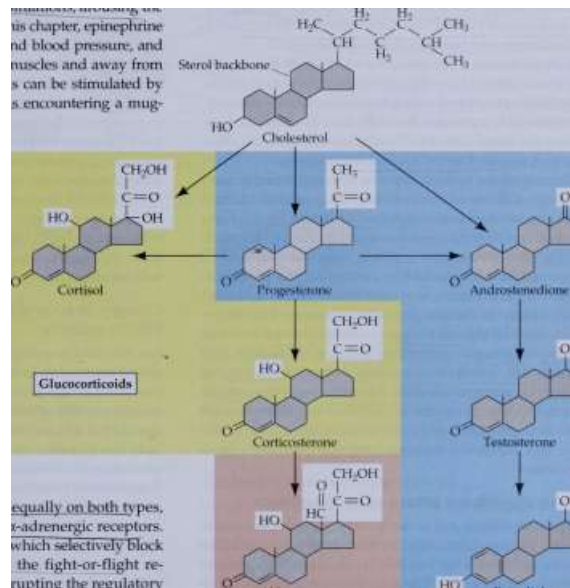
► The glucocorticoids influence blood glucose concentrations as well as other aspects of fat, protein, and carbohydrate metabolism.

► The mineralocorticoids influence the ionic balance of extracellular fluids.

► The sex steroids stimulate sexual development and reproductive activity.

The sex steroids are secreted in only negligible amounts by the adrenal cortex. They will be discussed in the next section, on gonadal hormones.

Sterol backbone



Aldosterone

HO

Estradiol

Mineralocorticoid

Sex steroids

41.12 The Corticosteroids Are Built from Cholesterol

Side groups on the sterol backbone give different properties to the different corticosteroid hormones. This simplified outline of steroid biosynthesis leaves out many intermediate steps.

726 CHAPTER FORTY-ONE

(Aldosterone, the main mineralocorticoid, stimulates the kidney to conserve sodium. If the adrenal glands are removed from an animal, it must have sodium added to its diet, or its sodium will be depleted and it will die. One human patient with a nonfunctional adrenal gland compensated by salting her food heavily and, in addition, ate a 60-pound block of salt in the course of a year. - _

The main glucocorticoid, cortisol, is critical for mediating the body's response to stress. As we have seen, your immediate reaction to a frightening situation is stimulated by your nervous system and by the release of epinephrine. This fight-or-flight response ensures that your muscles will have enough oxygen and glucose to fuel your escape. You have a limited amount of blood glucose, however, and you need to conserve it for your muscles and your brain. Within minutes of the frightening stimulus, blood cortisol level rises. Cortisol stimulates cells not critical for your escape to decrease their use of blood glucose and shift instead to utilizing fats and proteins for energy. This is not a time to feel sick, have allergic reactions, or heal wounds, so cortisol also blocks immune system reactions. This is why cortisol is useful for reducing inflammations and allergies.

Cortisol release is controlled by ACTH from the anterior pituitary, which in turn is controlled by the hypothalamic adrenocorticotropic-releasing hormone. Because the cortisol response to a stressor has this chain of steps, each involving secretion, diffusion, circulation, and cell activation, it is much slower than the epinephrine response.

Turning off the cortisol response is as important as turning it on. A study of stress in rats showed that old rats could turn on their stress responses as effectively as young rats, but that they had lost the ability to turn them off as rapidly. As a result, they suffered from the well-known consequences of stress: ulcers, cardiovascular problems, strokes, impaired immune system function, and increased susceptibility to cancers and other diseases. Further research showed that turning off stress responses involves the long-loop negative feedback action of cortisol on cells in the brain, which causes a decrease in the release of an adrenocorticotropic-releasing hormone (see Figure 41.8). Repeated activation of this negative feedback mechanism leads to a gradual loss of cortisol-sensitive cells in the brain, and therefore a decreased ability to terminate stress responses.

The sex steroids are produced by the gonads

The gonads—the testes of the male and the ovaries of the female—produce hormones as well as gametes. Most of the gonadal hormones are steroids synthesized from cholesterol (see Figure 41.12). The male steroids are collectively called androgens, and the dominant one is testosterone. The female steroids are estrogens and progesterone. The dominant estrogen is estradiol.

The sex steroids have important developmental effects: They determine whether a fetus develops into a female or a male. (A fetus is the latter stage of an embryo; a human em-

bryo is called a fetus from the eighth week of pregnancy to the moment of birth.) After birth, the sex steroids control the maturation of the reproductive organs and the development and maintenance of secondary sexual characteristics, such as breasts and facial hair.

The sex steroids begin to exert effects in the human embryo in the seventh week of development. Until that time, the embryo has the potential to develop into either sex. In mammals and birds, the ultimate instructions for sex determination reside in the genes. In mammals, individuals that receive two X chromosomes normally become females, and individuals that receive an X and a Y chromosome normally become males (Figure 41.13). These genetic instructions are carried out through the production and action of the sex steroids, and the potential for error exists.

The presence of a Y chromosome normally causes the embryonic, undifferentiated gonads to begin producing androgens in the seventh week. In response to the androgens, the reproductive system develops into that of a male. If androgens are not produced at that time, female reproductive structures develop. In other words, androgens are required to trigger male development in humans. The opposite situation exists in birds: Male characteristics develop unless estrogens are present to trigger female development.

Occasionally the hormonal control of sexual development does not work perfectly, resulting in intersex individuals. The most extreme (but rare) case is a true hermaphrodite, who has both testes and ovaries. Pseudohermaphrodites have the gonads of one sex and the external sex organs of the other. For example, an XY fetus will develop testes, but if his tissues are insensitive to the androgens they produce because his androgen receptors do not function, the testes will remain within the abdomen, and the external sex organs and the secondary sexual characteristics of a female will develop.

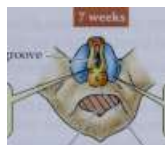
Changes in control of sex steroid production initiate puberty

Sex steroids have dramatic effects at puberty —the time of sexual maturation in humans. Sex steroids are produced at low levels by the juvenile gonads, but their production increases rapidly at the beginning of puberty—around the age of 12 to 13 years. Why does this sudden increase occur? In the juvenile, as in the adult, the production of sex steroids by the ovaries and testes is controlled by the anterior pituitary tropic hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which together are called the gonadotropins. The production of these tropic hormones is under the control of the hypothalamic gonadotropin-releasing hormone (GnRH). Prior to puberty, the gonads are capable of responding to gonadotropins, and the pituitary is capable of responding to GnRH. But prior to puberty the hypothalamus produces only very low levels of GnRH. Puberty is initiated by a reduction in the sensitivity of hypothalamic GnRH-producing cells to negative feedback from sex steroids and from gonadotropins. As a result, GnRH release increases, stimulating increased pro-

Undifferentiated embryo

Urethral groove

These folds of tissue will form the penis in the male or the labia minora in the female.



This tissue will form the scrotum in males or labia majora in females.

Tail (cut)

Anus

Androgens present

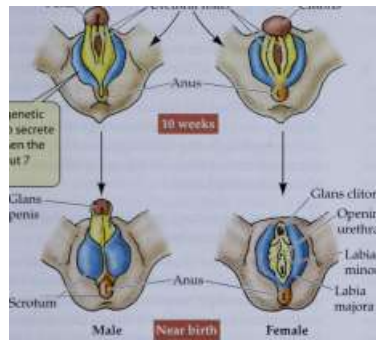
Androgens absent

Penis

-Urethral folds,

Clitoris

The testes of genetic males begin to secrete androgens when the embryo is about 7 weeks old.



Opening of urethra

Scrotum

Male

Female

Under the influence of androgens, a penis and scrotum form.

Without the influence of androgens, female external organs develop.

41.13 The Development of Human Sex Organs

The sex organs of early human embryos are similar. Male sex steroids (androgens) promote the development of male sex organs. Without androgen action, female sex organs form, even in genetic males.

duction of gonadotropins and hence increased production of sex steroids.

In females, increasing levels of LH and FSH at puberty stimulate the ovaries to begin producing the female sex hormones. The increased circulating levels of these hormones initiate the development of the traits of a sexually mature woman: enlarged breasts, vagina, and uterus; broad hips; increased subcutaneous fat; pubic hair; and the initiation of the menstrual cycle.

In the male, an increasing level of LH stimulates groups of cells in the testes to synthesize androgens, which in turn initiate the profound physiological, anatomical, and psychological changes associated with adolescence. The voice deepens, hair begins to grow on the face and body, and the testes and penis grow. Androgens also help skeletal muscles grow, especially when they are exercised regularly.

Natural muscle development can be exaggerated by both men and women who want to increase their maximum

ANIMAL HORMONES 727

strength in athletic competition if they take synthetic androgens—called anabolic steroids. However, anabolic steroids have serious negative side effects. In women, their use causes the breasts and uterus to shrink, the clitoris to enlarge, menstruation to become irregular, facial and body hair to grow, and the voice to deepen. In men, the testes shrink, hair loss increases, the breasts enlarge, and sterility can result. You can understand the causes of some of these side effects by considering the negative feedback effects of sex steroids on the production of LH and FSH. Other side effects are even more serious. Continued use of anabolic steroids greatly increases the risk of heart disease, certain cancers, kidney damage, and personality disorders such as depression, mania, psychoses, and extreme aggression. Most official athletic organizations, including the International Olympic Committee, ban the use of anabolic steroids.

Melatonin is involved in biological rhythms and photoperiodicity

The pineal gland is situated between the cerebral hemispheres of the brain on a little stalk. It produces the hormone melatonin from the amino acid tryptophan. In various vertebrates, melatonin is involved in biological rhythms and photoperiodicity. Photoperiodicity is the phenomenon whereby seasonal changes in day length cause physiological changes in animals. Many species, for example, come into reproductive condition when the days begin to get longer (Figure 41.14). Humans are not photoperiodic, but melatonin in humans may play a role in entraining the daily rhythm of physiological and behavioral activities to the daily cycle of light and dark.

The release of melatonin by the pineal occurs in the dark and therefore marks the length of the night. Exposure to light inhibits the release of melatonin. The pineal of birds is directly sensitive to light, but in mammals, the light response is mediated through the eyes via a group of cells at the base of the brain, which generates a daily rhythm for many physiological functions of the body. We will learn more about this brain structure in Chapter 52.

The list of other hormones is long

We have discussed the major endocrine glands and the "classic" hormones in this chapter, but there are many hormones we have not mentioned. Examples include the hormones produced in the digestive tract that help organize the way the gut processes food (see Table 41.1 and Chapter 50). Even the heart has endocrine functions. When blood pressure rises and causes the walls of the heart to stretch, certain cells in the walls of the heart release atrial natriuretic hormone. This hormone

increases the excretion of sodium ions and water by the kidneys, thereby lowering blood volume and blood pressure. As we discuss the organ systems

728 CHAPTER FORTY-ONE

(«)

Winter

(long nights)

Light Dark Light

Summer (short nights)

Time of day

(b)



Winter hamster

Summer hamster

47.74 The Release of Melatonin Regulates Seasonal Changes

(a) Melatonin is released in the dark and inhibited by light exposure. The duration of daily melatonin release thus changes as the day length (photoperiod) changes, inducing dramatic seasonal physiological changes in some animals, (b) In winter these Siberian hamsters are white and are non-reproductive. In summer they are mottled brown and breed.

of the body in the chapters that follow, we will frequently mention hormones that their tissues produce, or hormones that control their functions.

Mechanisms of Hormone Action

The hormones we have discussed are released in very small quantities, yet they can cause large responses in cells or tissues all over the body, and these responses can be quite specific in different cells. For example, we have discussed the many dramatic effects of testosterone, yet its concentration in the blood of adult human males is only about 30-100 ng/ml. How can hormones in such tiny quantities have such strong and selective actions?

The actions of hormones depend on receptors and signal transduction pathways

The selective action of hormones is explained by the fact that only cells with appropriate receptors respond to a hormone. Also, in different types of cells, the receptors for a particular hormone can be linked to different response mechanisms. There are numerous signal transduction pathways in cells (see Chapter 15), and therefore the response of a cell to a hormone depends both on the receptors and on the signal transduction pathways that exist in that cell. Epinephrine, for example, acts through four different receptors and several signal transduction pathways to induce a wide variety of responses in different tissues (Table 41.3).

The strength of hormone action frequently results from signal transduction cascades that amplify the original signal, as described in Chapter 15. An example is the response of liver cells to epinephrine (see Figure 15.17). A single molecule of epinephrine binding to its receptor on a liver cell can result in that liver cell releasing millions of molecules of glucose into the blood.

Hormone receptors are either on the cell surface or in the cell interior

Hormones can be classified as lipid-soluble or water-soluble, and that classification relates to where their receptors are. Lipid-soluble hormones, such as the steroid hormones and thyroxine, can diffuse through plasma membranes, and therefore their receptors are inside the cell, either in the cytoplasm or in the nucleus. In most cases, the complex formed by the lipid-soluble hormone and its receptor acts by altering gene expression in the cell, as described in Chapter 15.

Water-soluble hormones cannot readily pass through plasma membranes, and their receptors are on the cell surface. Water-soluble hormones include peptides such as the hypothalamic releasing hormones, proteins such as insulin and glucagon, and some other kinds of molecules, such as epinephrine and norepinephrine.

The cell surface receptors of water-soluble hormones are large glycoprotein complexes with three domains: a binding domain projecting beyond the outside of the plasma membrane, a transmembrane domain that anchors the receptor in the membrane, and a catalytic domain that extends into the

Diverse Actions of the Hormone Epinephrine

TISSUE

RECEPTOR

SIGNAL TRANSDUCTION PATHWAY

ACTION

Arterioles in skin and gut Arterioles in leg muscles Heart muscle Liver cells

Fat cells Pancreas

cytoplasm of the cell. The catalytic domain initiates cell responses by directly or indirectly activating protein kinases or protein phosphatases. In the direct pathway the catalytic domain changes its shape and becomes capable of phosphorylating tyrosine residues on protein kinases or phosphatases, thus changing their enzymatic activities. In the indirect pathway, the catalytic domain stimulates one of the several second messenger pathways that were described in Chapter 15.

Regulation of hormone receptors controls sensitivity of cells to hormones

We learned above that the release of hormones can be under feedback control, usually negative feedback control. Similarly, the abundance of receptors for a hormone can be under feedback control. In some cases, continuous high levels of a hormone can decrease the number of its receptors, in a process known as downregulation.

An example of downregulation is type II diabetes mellitus, also called insulin-independent diabetes, or sometimes adult-onset diabetes, because it occurs more frequently in adulthood than does type I diabetes mellitus, which is due to a lack of insulin and is usually diagnosed in childhood. Type II diabetes is distinguished by elevated levels of circulating insulin, but a loss of receptors. Although genetic factors are likely to be involved, a possible immediate cause of the disease is an overstimulation of pancreatic release of insulin by excessive carbohydrate intake, which leads to downregulation of the insulin receptors.

Upregulation of receptors is a positive feedback mechanism, and is less common. One example, however, is the monthly ovarian cycle of human females, whereby an ovum matures and is released. As we will learn in the next chapter, the maturation of the ovum and its associated cells is under the control of FSH. Early in this process, FSH causes the cells associated with the ovum to increase their production of FSH receptors. This upregulation of FSH receptors accelerates the maturation process stimulated by FSH.

Responses to hormones can vary greatly

So far in this chapter we have discussed two mechanisms for regulating physiological responses to hormones: controlling the amount of hormone released, and controlling the availability of receptors. Many other factors can influence physiological responses to hormones; therefore, it is valuable to be able to characterize these responses. One way is to construct a dose-response curve.

47.75 Dose-Response Curves Quantify Response to a Hormone

Between the threshold and maximum values, a dose-response curve frequently has a sigmoid or S shape. Anything that changes the responsiveness of a system—number of receptors in target cells, presence of enzymes and/or substrate, for example—affects the position of the curve.

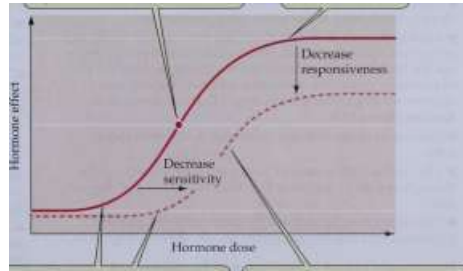
To create such a curve, cells, tissues, organs, or even a whole animal are experimentally treated with different amounts of a hormone and the response is measured. The response is then plotted on the y axis of a graph, and the amount of the hormone used is plotted on the x axis (Figure 41.15). The resulting dose-response curve shows the threshold dose of the hormone necessary to get a response and the dose of the hormone that produces the maximum response. Between these two extreme values, the curve frequently has a sigmoid or S shape. The hormone dose that stimulates half the maximum response indicates the sensitivity of the cell, tissue, organ, or animal to the hormone.

Anything that changes the responsiveness of a system to a hormone is reflected in the dose-response curve. A change in the number of receptors in the responding cells, for example, can result in changes in threshold and in sensitivity. Changes in signaling pathways, rate-limiting enzymes, or the availability of substrates or cofactors can result in changes in the maximum response. Dose-response curves are valuable tools for studying the many factors that influence hormone-mediated processes.

Responses to hormones can also vary in their time course. Hormones are not simple on-off switches, and the time course over which it acts is an important characteristic of a hormone. This characteristic can be measured by the hormone's half-life in the blood. Soon after endocrine cells are stimulated to secrete their hormone, the hormone reaches its maximum concentration in the blood. By taking subsequent blood samples, researchers can determine how long it takes for the circulating hormone to drop to half of that maximum concentration.

The fight-or-flight response to epinephrine is relatively quick in its onset and termination, and the half-life of epinephrine in the blood is only 1 to 3 minutes. The actions of

The dose that stimulates half the maximum response indicates the organism's sensitivity to the hormone. At this dose, the hormone reaches its maximum response.



The minimum dose of hormone that elicits a response is called the threshold.

Changes in such factors as the number of receptors in the responding cells, rate-limiting enzymes, or the availability of substrate can change sensitivity and responsiveness of the system.

730 CHAPTER FORTY-ONE

other hormones, such as Cortisol or thyroxine, are expressed over much longer periods, and their half-lives are on the order of days.

Once a hormone has been released, its half-life is partially determined by processes of degradation and elimination. Hormones are enzymatically degraded in the liver, then they are removed from the blood in the kidney and excreted in the urine. The presence of hormones or their breakdown products in the urine is the reason that urine samples can provide important information in clinical tests. In addition, most hormones are taken up and degraded by the very cells on which they act, so that they are not available to continually activate receptors.

Another factor that influences the half-life of a hormone is its ability to leave the blood. Some hormones, such as epinephrine, circulate as free molecules, but many circulate bound to carrier proteins. The extent to which hormones are bound to carrier proteins limits their ability to diffuse out of the blood to reach their target cells, to be degraded in the liver, or to be excreted by the kidney.

For example, when the mineralocorticoid aldosterone is released, about 15 percent of it binds to carrier proteins, and its half-life is 25 minutes. In contrast, when thyroxine is released, almost 100 percent of it binds to carrier proteins, and thyroxine has a half-life of 6 days. This variation in the time course of hormone responsiveness allows hormone signaling systems to have temporal characteristics that match their functions.

Of course, the nature of the target cell response to a hormone is also a factor in determining the time course of hormone action. For a hormone that stimulates a developmental effect, the time course of hormone action can be months, years, and even a lifetime. A very good example of a long-term process regulated primarily by hormones is animal reproduction, the topic of the next chapter.

Chapter Summary

Hormones and Their Actions

- ▶ Endocrine cells secrete chemical messages called hormones, which bind to receptors on or in target cells.
- ▶ Most hormones diffuse through the extracellular fluids and are picked up by the blood, which distributes them throughout the body. Some hormones diffuse to targets near the site of secretion. Autocrine hormones influence the cell that secretes them; paracrine hormones influence nearby cells. Review Figure 41.1
- ▶ Hormones cause different responses in different target cells.
- ▶ The chemical structures of hormones have changed little through evolution, but their functions have changed dramatically.
- ▶ Hormones may be secreted by single cells or by cells organized into discrete endocrine glands. Review Figure 41.2

Hormonal Control of Molting and Development in Insects

- ▶ Insects must molt their exoskeletons to grow. Two diffusible substances, brain hormone and ecdysone, control molting. Review Figure 41.3
- ▶ Juvenile hormone, another diffusible substance, prevents maturation so that juvenile instars molt into bigger juvenile instars. When the level of juvenile hormone falls low enough, the juvenile molts into the adult form.
- ▶ Some insects, such as butterflies, go through complete metamorphosis. When juvenile hormone drops to a low level, the larval form becomes a pupa. Because no juvenile hormone is secreted during pupation, the pupa molts into an adult. Review Figure 41.4

Vertebrate Endocrine Systems

- ▶ Vertebrates have nine endocrine glands that secrete many hormones. Review Figure 41.2, Table 41.1
- ▶ The pituitary gland is divided into two parts. The anterior pituitary develops from embryonic mouth tissue; the posterior pituitary develops from the brain.
- ▶ The posterior pituitary secretes the neurohormones vasopressin and oxytocin. Review Figure 41.5
- ▶ The anterior pituitary secretes tropic hormones (thyrotropin, adrenocorticotropin, and two gonadotropins), as well as growth hormone, prolactin, melanocyte-stimulating hormone, endorphins, and enkephalins.
- ▶ The anterior pituitary is controlled by neurohormones produced by cells in the hypothalamus and transported through portal blood vessels to the anterior pituitary. Review Figure 41.7, Table 41.2
- ▶ Hormone release in the

hypothalamus/pituitary/endocrine gland axis is controlled by many feedback loops. Review Figure 41.8

- ▶ The thyroid gland is controlled by thyrotropin and secretes thyroxine, which controls cell metabolism. Goiter can be associated with too little or too much thyroxine. Review Figure 41.9
- ▶ The level of calcium in the blood is regulated by two hormones. Calcitonin, produced by the thyroid, lowers blood calcium. Parathormone, produced by the parathyroid glands, raises it. Review Figure 41.10
- ▶ The pancreas secretes three hormones. Insulin stimulates glucose uptake by cells and lowers blood glucose, glucagon raises blood glucose, and somatostatin slows the rate of nutrient absorption from the gut.
- ▶ The adrenal gland has two portions, one within the other. The hormones of the adrenal medulla, epinephrine and norepinephrine, stimulate the liver to supply glucose to the blood, as well as other fight-or-flight reactions. Review Figure 41.11

► The adrenal cortex produce three classes of corticosteroids: glucocorticoids, mineralocorticoids, and small amounts of sex steroids. Review Figure 41.12

► Aldosterone is a mineralocorticoid that stimulates the kidney to conserve sodium and to excrete potassium.

► Cortisol is a glucocorticoid that decreases glucose utilization by most cells.

► Sex hormones (androgens in males, estrogens and progesterone in females) are produced by the gonads in response to tropic hormones. Sex hormones control sexual development, secondary sexual characteristics, and reproductive functions. Review Figure 41.13

► The pineal hormone melatonin is involved in controlling biological rhythms and photoperiodism. Review Figure 41.14

Mechanisms of Hormone Action

► The responses of a cell to a hormone depend on what receptors it has and what signal transduction pathways those receptors activate. Review Table 41.3

ANIMAL HORMONES 731

► The receptors for water-soluble hormones are on the cell surface, and the receptors for lipid-soluble hormones are inside the cell.

► The sensitivity of a cell to hormones can be altered by up-or downregulation of the receptors in that cell.

► The sensitivity and time course of a response to a hormone depend on many factors, including receptor numbers, properties of signal transduction pathways, the actions of other hormones, binding of the hormone to carrier proteins, and elimination of the hormone through degradation and excretion.

► Important tools for characterizing hormone action are dose-response curves and measurements of half-life. Review Figure 41.15

For Discussion

1. Explain how both hyperthyroidism and hypothyroidism can cause goiter. Refer to the roles of the hypothalamus and the pituitary in your answer.
2. In the 1960s, women who showed signs of premature labor were sometimes treated with progestins (substances that have progesterone activity) to inhibit contractions. The female children of these women tended to be "tomboys." In recent years many of these offspring have claimed that they feel more male than female and have therefore undergone sex change operations. Can you suggest an explanation for this phenomenon? (Hint: Review Figure 41.12.)
3. Various side effects of anabolic steroid use were mentioned in this chapter. Some of these effects are due to the direct action of the steroid, but others are due to the negative feedback action of the steroid. Discuss an example of each and explain possible mechanisms.
4. The time course of hormone action can vary over a broad range. Compare the characteristics you would expect of a hormone signaling system that controls a short-term process, such as digestive functions, with the characteristics you would expect of a hormone signaling system that controls a long-term process, such as a developmental process.



Animal Reproduction



Natural selection has created some amazing and bizarre adaptations, but among the most unusual and diverse are the methods some animals use to reproduce. Just as "unmanned" submersibles are used in deep ocean exploration, some species of polychaete worms use "unwormed" submersibles to reproduce. The adults of these marine worms live in burrows on the ocean floor or in reefs. Predators make it dangerous for them to leave their burrows to seek a mate, and if they simply released their eggs and sperm at the mouth of the burrow, they would have a poor chance of successful fertilization. So both males and females develop specialized body segments that form at the worm's posterior end and become stuffed with sperm or eggs. These segments develop sensory organs but no mouth or gut, since they will not need to feed.

When the time is right—full moon for some species, new moon for others—these "sex-cell transporters" break loose from the main body of the worm, leave the burrow, swim up into the water column, swarm with more of their kind, and release their sperm or eggs. The sex-cell transporters die soon after they release their cargo. Union of sperm and eggs takes place in the water column, and fertilized eggs may drift a long way before they descend to the ocean floor and develop into adult worms. But in many places, the native people know when and where the sex-cell transporters will swarm, and people harvest them for food.

In this chapter you will learn how animals reproduce. We first examine asexual mechanisms of reproduction, in which only a single parent is involved, and then turn to sexual reproduction, which requires two parents. Sexually reproducing organisms produce haploid sex cells—sperm and eggs—through the process of meiosis. An egg and a sperm must unite through the process of fertilization to create a new diploid individual.

As we will see, much of the diversity in reproductive systems is in mechanisms for getting sperm and eggs together. This chapter, however, focuses the most attention on the anatomy, function, and endocrine control of the human re-

Feasting on Sex Cells

During the final quarter of November's moon, the people of Samoa and Fiji harvest the reproductive segments of the palolo worm, *Eunice viridis*. The adult worms release specialized reproductive vehicles into the ocean according to a precise cycle that native people have understood for centuries. The protein-rich worm segments are prepared by roasting or frying and are eaten as a delicacy.

productive system. This information will allow us to understand the technologies we use both to limit and to overcome infertility. We end the chapter with a discussion of sexual health and sexually transmitted diseases.

Asexual Reproduction

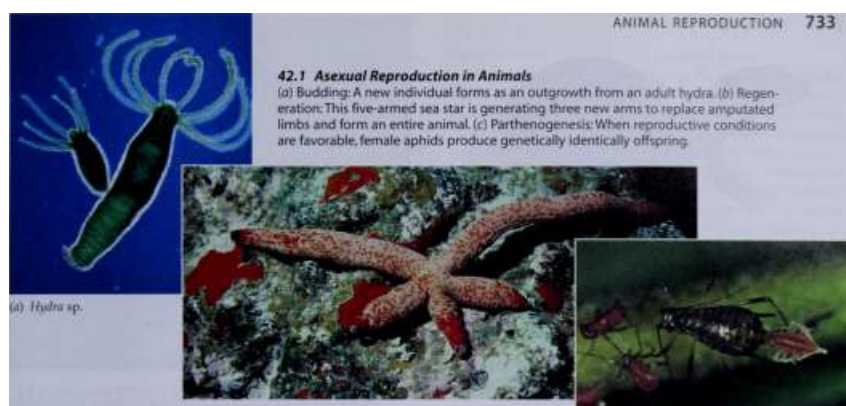
Sexual reproduction is a nearly universal trait of animals, although many species can reproduce asexually as well. Offspring produced asexually are genetically identical to one another and to their parents. Asexual reproduction is highly efficient because there is no mating. Mating requires energy, involves risks, and requires that resources be devoted to a large population of males, who do not produce offspring. Asexual populations can use resources efficiently because all individuals in the population can convert resources to offspring. However, asexual reproduction does not generate genetic diversity, and this can be a disadvantage in changing environments. As we learned in Chapter



ANIMAL REPRODUCTION

42.1 Asexual Reproduction in Animals

(a) Budding: A new individual forms as an outgrowth from an adult hydra, (b) Regen-eration: This five-armed sea star is generating three new arms to replace amputated limbs and form an entire animal, (c) Parthenogenesis: When reproductive conditions are favorable, female aphids produce genetically identical offspring.



(b) *Linckia* sp.

(c) *Macrosiphum rosae*

21, genetic diversity enables natural selection to shape adaptations in response to environmental change.

A variety of species, mostly invertebrates, reproduce asexually. They tend to be species that are sessile and cannot search for mates, or species that live in sparse populations and rarely encounter potential mates. Furthermore, asexually reproducing species are likely to be found in relatively constant environments, in which the potential for rapid evolutionary change is not as important as in more variable environments.

There are three common modes of asexual reproduction:

- ▶ Budding, in which new individuals form by mitotic cell division.
- ▶ Regeneration, in which a piece or section of an organism can generate an entire new individual.
- ▶ Parthenogenesis, in which individuals develop from unfertilized eggs.

Budding and regeneration produce new individuals by mitosis

Many simple multicellular animals produce offspring by budding; new individuals form as outgrowths of the bodies of older animals. These buds grow by mitotic cell division, and the cells differentiate before the buds break away from the parent (Figure 42.1a). The bud is genetically identical to the parent, and it may grow as large as the parent before it becomes independent.

Regeneration is usually thought of as the replacement of damaged tissues or lost limbs, but in some cases pieces of an organism can regenerate complete individuals. In a classic experiment demonstrating regeneration, a sponge is pushed through a cloth mesh, producing many little clusters of cells. Each cluster grows into a small but complete sponge. The ability of sponges to regenerate was used off the coast of Florida to restore the commercial bath-sponge

fishery, which was endangered by overfishing. Echinoderms also have remarkable abilities to regenerate. If sea stars are cut into pieces, each piece that includes a portion of the central disc grows into a new animal (Figure 42.1b).

Regeneration frequently results when an animal is broken by an outside force. A storm, for example, can cause a heavy surf that breaks colonial cnidarians such as corals. Pieces broken off the colony can regenerate into new colonies. In some species, the breakage occurs in the absence of external forces. Some species of segmented marine worms related to the ones we discussed at the beginning of this chapter develop segments with rudimentary heads bearing sensory organs, then break apart. Each fragmented segment forms a new worm.

Parthenogenesis is the development of unfertilized eggs

Not all eggs have to be fertilized to develop. A common mode of asexual reproduction in arthropods is the development of offspring from unfertilized eggs. This phenomenon, called parthenogenesis, also occurs in some species of fish, amphibians, and reptiles. Most species that reproduce parthenogenetically also engage in sexual reproduction or sexual behavior.

The aphids that can rapidly populate your rosebushes in the spring and summer reproduce parthenogenetically while conditions are favorable (Figure 42.1c). Some of the unfertilized eggs laid in spring and summer develop into male aphids, others into females. As conditions become less favorable, the aphids mate, and the females lay fertilized eggs. These eggs do not hatch until the following spring, and they yield only females.

In some species, parthenogenesis is part of the mechanism that determines sex. For example, in many hymenopterans (ants, and most species of bees and wasps), males develop from unfertilized eggs and are haploid.

734 CHAPTER FORTY-TWO Lizard acting as ♀

Lizard acting as O*



(b)

Cnemidophorus uniparens

42.2 Sexual Behavior May Be Required for Asexual Reproduction

(a) Parthenogenetic whiptail lizards are all female, but take turns acting the male role in reproductive behavior, (b) The stage of the ovarian cycle determines the role an individual plays.

Females develop from fertilized eggs and are diploid. Most females are sterile workers, but a select few become fertile queens. After a queen mates, she has a supply of sperm that she controls, enabling her to produce either fertilized or

unfertilized eggs. Thus the queen determines when and how much of the colony resources are expended on males. Parthenogenetic reproduction in some species requires sexual activity even though this activity does not fertilize eggs. The eggs of parthenogenetically reproducing ticks and mites, for example, develop only after the animals have mated, even though the eggs remain unfertilized. One case that has been investigated by David Crews and his students at the University of Texas is parthenogenetic reproduction in a species of whiptail lizard. There are no males in this species, but females act as males, engaging in all aspects of courtship display and mating, even though no sperm are produced or transferred (Figure 42.2). Whether a specific female acts as a female or as a male depends on her hormonal state at the time, but sexual activity is required to stimulate ovulation.

Sexual Reproduction

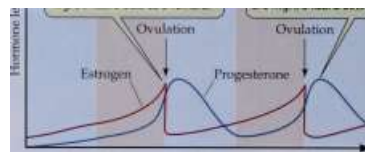
A large portion of the time and energy budgets of sexually reproducing animals goes into sexual behavior, which exposes them to predation, can result in physical damage, and detracts from other useful activities such as feeding, building secure living places, and caring for existing offspring. In spite of all of these disadvantages, there is an overwhelming evolutionary advantage to sexual reproduction: It produces genetic diversity.

Sexual reproduction requires the joining of two haploid sex cells to form a diploid individual. These haploid cells, or gametes, are produced through gametogenesis, a process that involves meiotic cell divisions. Two events in meiosis contribute to genetic diversity: crossing over of homologous chromosomes, and the independent assortment of chromo-

Acts as a female male female male

When its estrogen levels are high, a lizard acts as a female.

When its progesterone levels are high, a lizard acts as a male.



Time

somes. Both of these genetic phenomena were described in Chapter 10.

Mating behavior also contributes to genetic diversity in sexually reproducing species. The genetic variation in the gametes of a single individual and the genetic variation between any two parents produce an enormous potential for genetic variation between any two offspring of a sexually reproducing pair of individuals. This genetic diversity is the raw material for natural selection; thus evolutionary change in sexually reproducing animals can be quite rapid.

There are three fundamental phenomena of sexual reproduction in animals:

- Gametogenesis (making sex cells)
- Mating (getting sex cells together)
- Fertilization (getting sex cells to fuse)

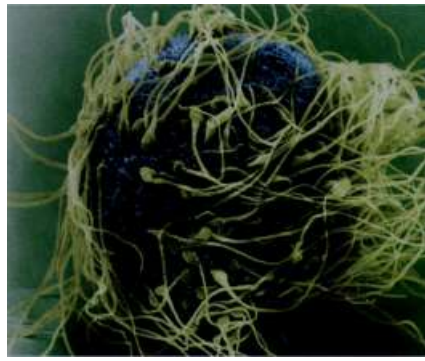
There is not a great deal of diversity in gametogenesis when we compare different groups of animals. Processes of fertilization are also rather similar in widely different species. Therefore, although the discussion of gametogenesis that follows is primarily derived from information from mammals, the facts would not be terribly different if we focused on a different group of animals.

Mating, on the other hand, shows incredible evolutionary diversity. Our discussion of mating in this chapter will focus on a few specific examples as representative of the fascinating diversity that exists.

Eggs and sperm form through gametogenesis

Gametogenesis occurs in the primary sex organs, the gonads, which are testes (singular testis) in males and ovaries in females. The tiny gametes of males, called sperm, are motile and move by beating their flagella. The much larger female gametes are eggs, or ova (singular ovum), and are nonmotile (Figure 42.3).

Gametes are produced from germ cells, which have their origin in the earliest cell divisions of the embryo and remain distinct from the rest of the body. All the rest of the cells of the embryo are called somatic cells. Germ cells are sequestered in the body of the embryo until its gonads begin to form. The germ cells then migrate to the gonads, where they take up residence and proliferate by mitosis,



42.3 Gametes Differ in Size

Mammalian sperm (white) are much smaller than the mammalian egg (blue), as illustrated by this artificially colored micrograph of human fertilization.

42.4 Gametogenesis

(a) Diploid spermatogonia develop into haploid spermatids. Spermatids differentiate into sperm. (6) Diploid oogonia develop into haploid secondary oocytes, which mature into ova.

ANIMAL REPRODUCTION 735

producing oogonia (singular oogonium) in females and spermatogonia (singular spermatogonium) in males. Oogonia and spermatogonia, which are diploid, multiply by mitosis in turn, eventually producing primary oocytes and primary spermatocytes, which are still diploid cells.

Meiosis, the next step in gametogenesis, reduces the chromosomes to the haploid number, and these haploid cells mature into sperm and ova. (You may want to review the discussion of meiosis in Chapter 9 before reading further.) Although the steps of meiosis are very similar in males and females, there are some significant differences in gametogenesis.

spermatogenesis produces sperm. Primary spermatocytes undergo the first meiotic division to form secondary spermatocytes, which are haploid. The second meiotic division produces four haploid spermatids for each primary spermatocyte that entered meiosis. In mammals, these cells remain connected by cross-bridges of cytoplasm after each division (Figure 42 Aa).

The reason that mammalian spermatocytes remain in cytoplasmic contact throughout their development probably is the asymmetry of sex chromosomes in the males of

Spermatids (ri)

Differentiation and maturation into gametes

Sperm cells (n)

(a) Spermatogenesis

Male germ cell (2m)

Spermatogonium (2n)

Primary

spermatocyte

{In)

Secondary spermatocytes (n)



The first meiotic division produces haploid cells.



.Cytoplasmic bridge



First meiotic division

Second meiotic division

Spermatids, each of which is different genetically, will differentiate into individual sperm.

(b) Oogenesis

Female germ cell ($2n$)

Oogonium ($2n$)

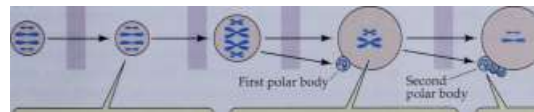
Primary oocyte ($2n$)

Differentiation and growth

Secondary oocyte (n)

Ootid (\gg)

Ovum (egg) (n)



Polar bodies degrade



Diploid oogonia develop into larger primary oocytes that grow and accumulate materials and energy.

The first meiotic division produces a haploid secondary oocyte and a small, adjacent, nucleus-containing polar body.

The second meiotic division produces another polar body and the haploid egg (the first polar body may also divide at this time).

736 CHAPTER FORTY-TWO

most species. Half of the secondary spermatocytes receive an X chromosome, the other half a Y chromosome. The Y chromosome contains fewer genes than the X chromosome, and apparently some of the products of genes not included in the Y chromosome are essential for spermatocyte development. By remaining in cytoplasmic contact, all spermatocytes can share the gene products of the X chromosomes, even though only half of them have an X chromosome.

Just after being produced by meiosis, a spermatid bears little resemblance to a sperm. Through further differentiation, it will become compact, streamlined, and motile. We will look at the differentiation of human sperm in more detail below.

Oogenesis produces eggs. Oogonia, like spermatogonia, proliferate through mitosis. The resulting egg precursor cells differentiate into primary oocytes, which immediately enter prophase of the first meiotic division. In many species, including humans, the development of the oocyte is arrested at this point, and may remain so for days, months, or years. In contrast, there is no arrest in the development of male gametes; the process goes steadily to completion once the primary spermatocyte has differentiated. In the human female, as we will see, some primary oocytes may remain in arrested prophase I for 50 years!

During this prolonged prophase I, or shortly before it ends, the primary oocyte undergoes its major growth phase. It grows larger due to increased production of ribosomes, RNA, cytoplasmic organelles, and energy stores. At this time, the primary oocyte acquires all of the energy, raw materials, and RNA that the egg will need to survive its first cell divisions after fertilization. In fact, the nutrients in the egg will have to nourish the embryo until it is either nourished by the maternal system or can feed on its own.

When a primary oocyte resumes meiosis, its nucleus completes the first meiotic division near the surface of the cell. The daughter cells of this division receive grossly unequal shares of cytoplasm. This asymmetry represents another major difference from spermatogenesis, in which cell divisions apportion cytoplasm equally. The daughter cell that receives almost all of the cytoplasm becomes the secondary oocyte, and the one that receives almost none forms the first polar body (see Figure 42.4b).

The second meiotic division of the large secondary oocyte is also accompanied by an asymmetrical division of the cytoplasm. One daughter cell forms the large, haploid ootid, which eventually differentiates into a mature ovum, and the other forms the second polar body. Polar bodies degenerate, so the end result of oogenesis is that each primary oocyte produces only one mature egg. However, that egg is a very large, well-provisioned cell.

A second period of arrested development occurs after the first meiotic division forms the secondary oocyte. The egg may be expelled from the ovary in this condition. In many species, including humans, the second meiotic division is not completed

until the egg is fertilized by a sperm.

A single body can function as both male and female

Sexual reproduction requires both male and female haploid gametes. In most species, these gametes are produced by individuals that are either male or female. Species that have male and female members are called dioecious (from the Greek for "two houses"). In some species, a single individual may possess both female and male reproductive systems. Such species are called monoecious ("one house") or hermaphroditic.

Almost all invertebrate groups have hermaphroditic species. An earthworm is an example of a simultaneous hermaphrodite, meaning that it is both male and female at the same time. When two earthworms mate, they exchange sperm, and as a result, the eggs of each are fertilized (see Figure 31.24b). Some animals are sequential hermaphrodites, meaning that individuals function as a male or as a female at different times in their lives.

What is the selective advantage of hermaphroditism? Some simultaneous hermaphrodites have a low probability of meeting a potential mate. An example is a parasitic tapeworm. Even though it may be large and cause lots of trouble for its host, it may be the only tapeworm in the host. Tapeworms can fertilize their own eggs. Most simultaneous hermaphrodites must mate with another individual, but since each member of the population is both male and female, the probability of encountering a possible mate is double what it would be in monoecious species.

Sequential hermaphroditism can reduce the possibility of inbreeding among siblings by making them all the same sex at the same time and therefore incapable of mating with one another. In a species in which only a few males fertilize all females, sequential hermaphroditism can maximize reproductive success by making it possible for an individual to reproduce as a female until the opportunity arises for it to function successfully as a male.

Anatomical and behavioral adaptations bring eggs and sperm together

Sexual reproduction requires that two haploid gametes join together to form a diploid zygote. The purpose of mating behavior is to get eggs and sperm close enough together that this process—called fertilization—can occur. Many anatomical and behavioral adaptations have evolved to support mating. The simplest distinction in mating systems is whether fertilization occurs externally or internally.

EXTERNAL FERTILIZATION REQUIRES AN AQUATIC HABITAT. In an

aquatic environment, animals can simply release their gametes into the water. External fertilization is common among simple aquatic animals that are not very mobile (Figure 42.5). These animals produce huge numbers of gametes. A female oyster, for example, may produce 100 million eggs in a year, and the number of sperm produced by a male oyster is astronomical.

But numbers alone do not guarantee that gametes will meet. Timing is also important. The reproductive activities

A



Acropora sp.

42.5 External Fertilization Is Common in Aquatic Species

External fertilization requires an aqueous environment. These staghorn corals are all releasing sperm-egg bundles into the oceans of Ningaloo Reef, Australia.

of the males and females of a population must be synchronized. Seasonal breeders may use day length, changes in temperature, or changes in weather to time their production and release of gametes. Social stimulation is also important. Sexual activity on the part of one member of a population can stimulate others to engage in mating.

Behavior can play an important role in bringing gametes together even when fertilization is external. Many species travel great distances to congregate with potential mates and release their gametes at the same time in a suitable environment. Salmon are an extreme example, traveling hundreds of miles to spawn in the stream where they hatched.

INTERNAL FERTILIZATION ENABLES TERRESTRIAL LIFE. Sperm Can

move only through liquid, and delicate gametes released into air would dry out and die. Terrestrial animals avoid these problems by engaging in internal fertilization.

Animals have evolved an incredible diversity of behavioral and anatomical adaptations to get male gametes into the female reproductive tract. As we saw above, gametogenesis occurs in the primary sex organs, the gonads. All of the additional anatomical components of an animal's reproductive system are called accessory sex organs. An obvious accessory sex organ in the male is a tubular structure called the penis, which enables the male to deposit sperm in the female's accessory sex organ, the vagina (or, in some species, the cloaca, a cavity common to the digestive, urinary, and reproductive systems). Accessory sex organs include a variety of glands, tubules, ducts, and other structures.

Copulation is the physical joining of the male and female accessory sex organs. Transfer of sperm in internal fertilization can also be indirect. Males of some species of mites and scorpions (among the arthropods) and salamanders

ANIMAL REPRODUCTION 737

deposit spermatophores — containers filled with sperm—in the environment. When a female mite finds a spermatophore, she straddles it and opens a pair of plates in her abdomen so that the tip of the spermatophore enters her reproductive tract and allows the sperm to enter. Some female salamanders use the lips of their cloacae to scoop up the spermatophore.

Male squid and spiders play a more active role in spermatophore transfer. The male spider secretes a drop containing sperm onto a bit of web; then, with a special structure on his foreleg, he picks up the sperm-containing web and inserts it through the female's genital opening. Male squid use one special tentacle to pick up a spermatophore and insert it into the female's genital opening.

Most male insects copulate and transfer sperm to the female's vagina through a tubular penis. The genitalia — external sex organs—of insects often have species-specific shapes that match in a lock-and-key fashion. This mechanism ensures a tight, secure fit between the mating pair during the prolonged period of sperm transfer. The males of some insect species have elaborate structures on their penises that can scoop sperm deposited by other males out of the female's reproductive tract.

The evolution of vertebrate reproductive systems parallels the move to land

The earliest vertebrates evolved in aquatic environments. The closest living relatives of those earliest vertebrates are modern-day fishes. They remain exclusively aquatic animals, and most practice external fertilization. The most primitive of the fishes, the lampreys and hagfishes, broadcast their gametes into the environment, as do many aquatic invertebrates. In most fishes, however, fertilization is more selective: Mating behaviors bring females and males into close proximity at the time of gamete release.

In some sharks and rays, certain fins have evolved into structures that hold the male and female together and enable sperm to be transferred directly into the female reproductive tract. This internal fertilization in sharks and rays has made it possible for the females of some species to enclose fertilized eggs in protective egg cases before depositing them in the environment.

Amphibians were the first vertebrates to live in terrestrial environments. They dealt with the challenge of a dry environment by returning to water to reproduce, as most amphibians still do today. Exceptions are the terrestrial salamanders that use spermatophores to transfer sperm, as mentioned earlier. The spermatophore provides a protective, non-desiccating environment for the sperm. Other amphibians, like most fishes, rely on sexual behavior to bring eggs and sperm together. Frog mating behavior is characterized by amplexus, a behavior in which a male grasps a female around the middle with his forelegs and holds on until she releases her egg mass, at which time he releases his sperm (Figure 42.6).

Reptiles were the first vertebrate group to solve the problem of reproduction in the terrestrial environment.

738 CHAPTER FORTY-TWO



Agalychnis saltator

42.6 Getting Sperm and Eggs Together

Fertilization in frogs is external, but amplexus—a behavior in which the male holds the female with his forelegs until she releases her egg mass—helps guarantee that sperm and eggs will get together.

Their solution, the shelled egg, is shared by birds (Figure 42.7). But the shelled egg created a new problem for fertilization: Sperm cannot penetrate the shell, so they have to reach the egg before the shell forms. Hence the need for internal fertilization and the evolution of the necessary accessory sex organs.

Male snakes and lizards have paired hemipenes, which can be filled with blood and thereby extruded from the male's cloaca to form intromittent organs. Only one hemi-



(a) *Chelonia mydas*

42.7 The Shelled Egg

The shelled egg was a major evolutionary step that allowed reptiles and birds to reproduce in the terrestrial environment, (a) A female green sea turtle deposits her eggs in the sand, (b) Because the terrestrial environment offers no water to bring sperm and egg together, fertilization must take place internally, as with these penguins.

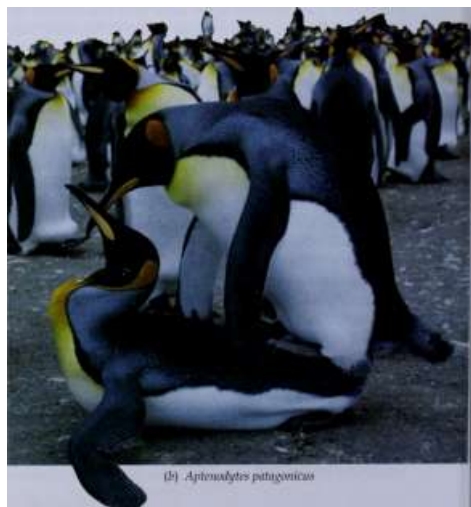
penis is inserted in the female's cloaca at a time. It is usually rough or spiny at the end to achieve a secure hold while sperm are transferred down a groove on its surface. Retractor muscles pull the hemipenis back into the male's body when mating is completed. Birds have erectile penises that channel sperm along a groove into the female cloaca.

All mammals use internal fertilization, but except for the monotremes, they have done away with the shelled egg. They keep the developing embryo in the female reproductive tract, at least through the early stages of development. Mammalian species differ enormously as to the developmental stage of the offspring at the time of birth.

Reproductive systems are distinguished by where the embryo develops

Two patterns of care and nurture of the embryo have evolved in animals: oviparity (egg bearing) and viviparity (live bearing). Oviparous animals lay eggs in the environment, and their embryos develop outside the mother's body. Oviparity is possible because eggs are stocked with abundant nutrients to supply the needs of the embryo.

Oviparous terrestrial animals such as insects, reptiles, and birds protect their eggs from desiccation with tough, waterproof membranes or shells. However, these egg coverings must be permeable to oxygen and carbon dioxide.



(b) *Aptenodytes patagonicus*



ANIMAL REPRODUCTION 739

Some oviparous animals engage in various forms of parental behavior to protect their eggs, but until the eggs hatch, the embryos depend entirely on the nutrients stored in the egg. The only oviparous mammalian species are the monotremes: the echidnas and the duck-billed platypus (see Figure 33.22).

Viviparous animals retain the embryo within the mother's body during its early developmental stages. Most mammals are viviparous. There are examples of viviparity in all other vertebrate groups except the crocodiles, turtles, and birds. Even some sharks retain fertilized eggs in their bodies and give birth to free-living offspring. But there is a big difference between viviparity in mammals and in other species. Mammals (except monotremes) have a specialized portion of the female reproductive tract, the uterus, that holds the embryo and enables it to derive nutrients from and deliver wastes to the maternal blood. In contrast, non-mammalian viviparous animals simply retain the fertilized eggs in the mother's body until they hatch. The embryos still receive their nutrition from the stores in the egg, so this reproductive adaptation is called ovoviviparity.

Among mammals there are various degrees of uterine adaptation. In marsupials, such as kangaroos and koalas, the uterus simply holds the embryo and has a limited capability for exchanging nutrients and wastes. Marsupials are born at a very early developmental stage, crawl into a pouch called a marsupium on the mother's belly, attach to a nipple, and complete development outside of the mother's uterus (see Figure 33.23). Mammals other than monotremes and marsupials are called eutherians. They are characterized by an intimate association of the blood supplies of mother and embryo in the walls of the uterus. We will now look at the reproductive system of eutherians in greater depth, using *Homo sapiens* as our model.



o Semen is ejaculated through the male copulatory organ, the penis.

The Human Reproductive System

So far we have seen a small sampling of the fascinating diversity of animal reproductive systems. In this section we describe the structures and functions of the male and female sex organs in eutherian mammals, specifically in human beings, and discuss hormonal regulation of both male and female systems.

Male sex organs produce and deliver semen

Semen is the product of the male reproductive system. Besides sperm, semen contains a complex mixture of fluids and molecules that support the sperm and facilitate fertilization. Sperm make up less than five percent of the volume of the semen.

Sperm are produced in the testes, the paired male gonads. In all mammals except bats, elephants, and marine mammals, the testes are located outside the body cavity in a pouch of skin, the scrotum (Figure 42.8). The optimal temperature for spermatogenesis in most mammals is slightly lower than the normal body temperature. The scrotum keeps the testes at this optimal temperature. Muscles in the scrotum contract in a cold environment, bringing the testes closer to the warmth of the body; in a hot environment they relax, suspending the testes farther from the body.

A testis consists of tightly coiled seminiferous tubules within which spermatogenesis takes place. Each tubule is lined with a stratified epithelium. Spermatogonia reside in the outer layers of this epithelium, and moving from these outer layers toward the lumen of the tubule, we find germ cells in successive stages of spermatogenesis (Figure 42.9). These germ cells are intimately associated with Sertoli cells, which protect them by providing a barrier between them and any noxious substances that might be circulating in the blood. Sertoli cells also provide nutrients for the developing sperm and are involved in the hormonal control

ro



Urinary bladder

o Seminal fluids are produced by the seminal vesicles, the prostate gland, and the bulbourethral gland.

Q Sperm are delivered to the urethra through the vas deferens, which joins the urethra behind the bladder.

f

Sperm are stored and mature in the epididymis.

GE5H

t



Urinary bladder

Pubic bone

Spongy, erectile tissue

Urethra

Glans penis

Foreskin

42.8 The Reproductive Tract of the Human Male

Front and side views of the male reproductive organs.

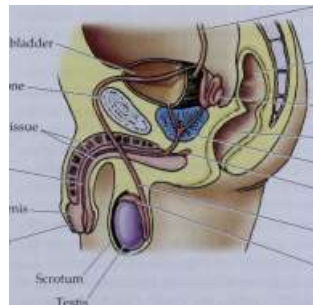
Ureter (from kidney)

Colon

Seminal vesicle

Rectum

Sperm are produced in the testes.



Prostate gland

Bulbourethral gland

Vas deferens

Epididymis

740 CHAPTER FORTY-TWO

Vas deferens

Epididymis

Testis



Each Sertoli cell envelops, nourishes, and protects developing sperm cells.

Nucleus of Sertoli cell f~

Sertoli cell

-^M

Seminiferous tubule

Cross section of seminiferous tubule

/ \

Sperm cells develop

continuously over the

great length of the
seminiferous tubules.

v v

Leydig cells in the tissue between seminiferous tubules produce male sex hormones.

Lumen of seminiferous tubule

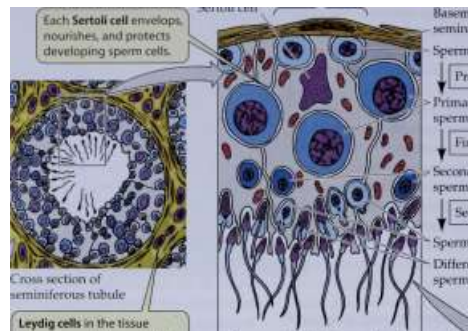
±

Mature sperm are shed into the lumen of the seminiferous tubule.



t 42.9 Seminiferous Tubules Are the Site of Spermatogenesis

Seminiferous tubules fill the testes of the human male. As sperm mature, they move from the wall of the tubule toward the center, where they are shed into the lumen of the tubule.



Basement membrane of seminiferous tubule

Spermatogonia

(In) develop into Spermatogonium ($2n$) l sperm cells (n).

Proliferation by mitosis

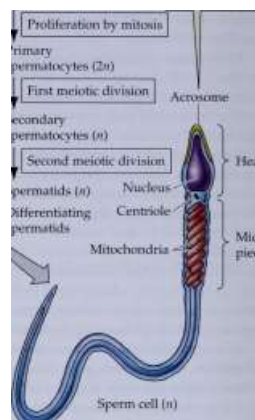
Primary spermatocytes ($2n$)

First meiotic division

Secondary spermatocytes (n)

Second meiotic division

> Head



Spermatids (n)

Differentiating spermatids

Mid-piece

Sperm cell (n)

of spermatogenesis. Between the seminiferous tubules are clusters of Leydig cells, which produce male sex hormones.

With completion of the second meiotic division, each primary spermatocyte has given rise to four spermatids (see Figure 42.4a), which develop into sperm as they continue to migrate toward the lumen of the seminiferous tubule. The nucleus in what will become the head of the sperm becomes compact, and the surrounding cytoplasm is lost (see Figure 42.9). A flagellum, or tail, develops. The mitochondria, which will provide energy for tail motility, become condensed into a midpiece between the head and the tail. A cap, called an acrosome, forms over the nucleus in the head of the sperm. The acrosome contains enzymes that enable the sperm to digest a path through protective layers surrounding the egg. Fully differentiated sperm are shed into the lumen of the seminiferous tubule.

From the tubules, sperm move into a storage structure called the epididymis, where they mature and become motile. The epididymis connects to the urethra by a tube called the vas deferens (plural vasa deferentia). The urethra originates in the bladder, runs through the penis, and opens to the outside of the body at the tip of the penis. It serves as the common duct for the urinary and reproductive systems (see Figure 42.8).

The penis and the scrotum are the male genitalia. The shaft of the penis is covered with normal skin, but the tip, or glans penis, is covered with thinner, more sensitive skin that is especially responsive to sexual stimulation. A fold of skin called the foreskin covers the glans of the human penis.

The cultural practice of circumcision removes a portion of the foreskin.

Sexual arousal triggers responses in the the autonomic nervous system that result in the erection of the penis. The vessels carrying blood into the penis dilate, and this increased blood flow fills and swells shafts of spongy, erectile tissue located along the length of the penis. The enlargement of these blood-filled cavities compresses the vessels that normally carry blood out of the penis. As a result, the erectile tissue becomes more and more engorged with blood. The penis becomes hard and erect, facilitating its insertion into the female's vagina.*

The culmination of the male sex act propels semen through the vasa deferentia and the urethra in two steps, emission and ejaculation. During emission, rhythmic contractions of the smooth muscles of the ducts containing sperm and of the accessory glands move sperm and the various secretions into the urethra at the base of the penis. Ejaculation, which follows emission, is caused by contractions of other muscles at the base of the penis surrounding the urethra. The rigidity of the erect penis allows these contractions to force the gelatinous mass of semen through the urethra and out of the body.

Once a climax has been achieved, the autonomic nervous system switches signaling and causes the vessels leading

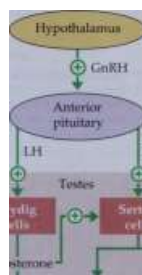
*In some species of mammals—but not humans—the penis contains a bone called the baculum or the os penis; however, even those species depend on erectile tissue for copulation.

Hypothalamic production of GnRH and pituitary production of LH are inhibited by high levels of circulating testosterone.

^"O+

>

—O+



«-O-».

<-<s>—

FSH

Leydig cells

Testosterone

Sertoli cells

Stimulate spermatogenesis

-(+)+ = Stimulates •O-> = Inhibits

Testosterone ©

Reproductive tract and other organs

Inhibin*

The hormone inhibin, produced by the Sertoli cells, inhibits GnRH and FSH production.

42.10 Hormones Control the Male Reproductive System

The male reproductive system is under hormonal control by the hypothalamus and the anterior pituitary.

into the penis to constrict. The resulting decrease in blood pressure in the erectile tissue relieves the compression of the blood vessels leaving the penis, and the erection declines.

The components of the semen other than sperm come from several accessory glands that contribute secretions to the urethra. A relatively small volume of fluid comes from the bulbourethral glands. This alkaline and mucoid secretion precedes other secretions; it neutralizes acidity in the urethra and lubricates the tip of the penis. About two-thirds of the volume of semen is seminal fluid, which comes from the seminal vesicles. Seminal fluid is thick because it contains mucus and protein. It also contains fructose, an energy source for the sperm, which are too small to carry much of their own fuel. Semen also carries a message for the female reproductive tract in the form of chemicals called prostaglandins. Prostaglandins stimulate rhythmic contractions in the female reproductive tract that help move the sperm up into the regions where fertilization can take place.

One-fourth to one-third of the volume of semen is a thin, milky fluid that comes from the prostate gland. Prostate fluid makes the uterine environment more hospitable to sperm. The prostate also secretes a clotting enzyme that works on the protein in seminal fluid to convert semen into a gelatinous mass. The prostate gland completely surrounds the urethra as it leaves the bladder. This gland tends to enlarge in men over 40 years of age, creating a condition known as benign prostate hyperplasia (BPH). A seriously enlarged prostate can block the urethra and make urination difficult. Unrelated to BPH, prostate cancer is the second most common cancer in men. It is relatively easy to diagnose, however, and is highly curable if detected early.

ANIMAL REPRODUCTION 741

Male sexual function is controlled by hormones

Spermatogenesis and maintenance of male secondary sexual characteristics depend on testosterone, which is produced by Leydig cells in the testes. In Chapter 41 we learned that increased production of testosterone at puberty is due to an increased release of gonadotropin-releasing hormone (GnRH) by the hypothalamus, which in turn stimulates cells in the anterior pituitary to increase their secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Figure 42.10). Negative feedback loops help to regulate testis functions.

The Leydig cells are stimulated by LH to produce testosterone. The rise in the level of testosterone in the prepubertal male causes the development of secondary sexual characteristics and the pubertal growth spurt, promotes increased muscle mass, and stimulates growth and maturation of the testes. If a male is castrated (has his testes removed) before puberty, he will not develop a deep voice, typical patterns of body hair, or a muscular build, and his external genitalia will remain childlike. Continued production of testosterone after puberty is essential to maintain secondary sexual characteristics and to produce sperm. Spermatogenesis is controlled by the influence of FSH and testosterone on Sertoli cells in the seminiferous tubules.

Female sex organs produce eggs, receive sperm, and nurture the embryo

When an egg matures, it is released from the ovary directly into the body cavity. But the egg can't go far. Each ovary is enveloped by the undulating, fringed opening of an oviduct (also known as a fallopian tube), which sweeps the egg into the tube (Figure 42.11). Cilia lining the oviduct propel the egg slowly toward the uterus, or *zomb*, which is a muscular, thick-walled cavity shaped like an upside-down pear. The uterus is where the embryo develops if the egg is fertilized. At the bottom of the uterus is an opening called the cervix, which leads into the vagina. Sperm are ejaculated into the vagina during copulation, and the fetus passes through the vagina during birth.

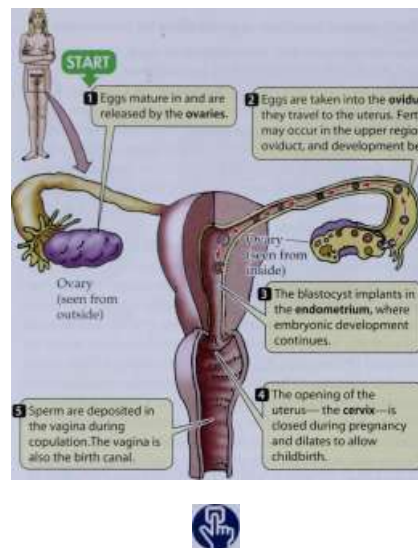
Two sets of skin folds surround the opening of the vagina and the opening of the urethra, through which urine passes. The inner, more delicate folds are the labia minora (singular labium minus); the outer, thicker folds are the labia majora (singular labium majus). At the anterior tip of the labia minora is the clitoris, a small bulb of erectile tissue that is the anatomical homolog of the penis. The clitoris is highly sensitive and plays an important role in sexual response. The labia minora and the clitoris become engorged with blood in response to sexual stimulation.

The opening of an infant female's vagina is partly covered by a thin membrane, the hymen. Eventually the hymen becomes ruptured by vigorous physical activity or first sexual intercourse; it can sometimes make first intercourse difficult or painful for the female.

To fertilize an egg, sperm swim and are propelled by contractions of the female reproductive tract up from the vagina, through the cervix, the uterus, and most of the

742 CHAPTER FORTY-TWO

o Eggs are taken into the oviducts, where they travel to the uterus. Fertilization may occur in the upper regions of the oviduct, and development begins.



42.1 7 The Reproductive Tract of the Human Female

Front and side views of the female reproductive organs.

|The blastocyst implants in the endometrium, where embryonic development continues.

Q Sperm are deposited in the vagina during copulation. The vagina is also the birth canal.

(The opening of the uterus—the cervix—is closed during pregnancy and dilates to allow childbirth.

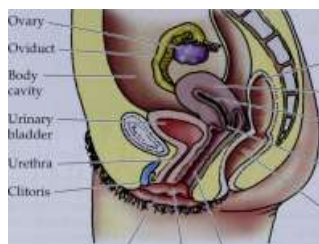
oviduct. The egg (actually a secondary oocyte in humans; see Figure 42. 4b) is fertilized in the upper region of the oviduct. Fertilization stimulates the completion of the second meiotic division, after which the haploid nuclei of the sperm and the egg can fuse to produce a diploid zygote nucleus. Still in the oviduct, the zygote undergoes its first few cell divisions to become a blastocyst. The blastocyst moves down the oviduct to the uterus, where it attaches itself to the epithelial lining, the endometrium. The endometrium and the cells of the uterine wall are stimulated by estrogen to proliferate and grow many new blood vessels in anticipation of receiving a blastocyst.

Once attached to the endometrium, the blastocyst burrows into it, a process called implantation, and forms a structure called the placenta. The placenta exchanges nutrients and waste products between the mother's blood and the baby's blood. If a blastocyst does not arrive in the uterus, the endometrium regresses or is sloughed off. Thus the female reproductive cycle actually consists of two linked cycles: an ovarian cycle that produces eggs and hormones, and a uterine cycle that creates an appropriate environment for the embryo should fertilization occur.

The ovarian cycle produces a mature egg

An ovarian cycle is about 28 days long in the human female,* but there is considerable variation among individu-

*Some mammals have ovarian cycles shorter than 28 days, others have longer ones. Rats and mice have ovarian cycles of about 4 days; many seasonally breeding mammals have only one ovarian cycle per year.



Colon

Uterus

Endometrium (lines uterus)

Cervix Rectum

Labia Labia Vagina majora minora

als. During the first half of the cycle, at least one primary oocyte matures into a secondary oocyte (egg) and is expelled from the ovary. During the second half of the cycle, cells in the ovary that were associated with the maturing oocyte develop endocrine functions and then regress if the egg is not fertilized. The progression of these events is shown diagrammatically in Figure 42.12.

At birth, a human female has about a million primary oocytes in each ovary. By the time she reaches sexual maturity, she has only about 200,000; the rest have degenerated. During a woman's fertile years, her ovaries will go through about 450 ovarian cycles, and during each of these cycles one oocyte will mature and be released. At about 50 years of age, she reaches menopause, the end of fertility, and may have only a few oocytes left in each ovary. Throughout a woman's life, oocytes are degenerating, and no new ones are produced.

Each primary oocyte in the ovary is surrounded by a layer of follicle cells. An oocyte and its follicle cells constitute the functional unit of the ovary, the follicle. Between puberty and menopause, six to twelve follicles begin to mature each month. In each of these follicles, the oocyte enlarges and the surrounding cells proliferate. After about a week, one of these follicles is larger than the rest and continues to grow, while the others cease to develop and shrink. In the enlarged follicle, the follicle cells nurture the growing egg, supplying it with nutrients and with macro-molecules and proteins that it will use in early stages of development if it is fertilized.

After 2 weeks of follicular growth, ovulation occurs— the follicle ruptures, and the egg is released. Following ovulation, the follicle cells continue to proliferate and form a mass of endocrine tissue about the size of a marble. This

FTI ^ 42.12 The Ovarian Cycle

The ovarian cycle progresses from the development of a follicle to ovulation and finally to growth and degeneration of the corpus luteum. The micrograph shows a mature mammalian follicle; the oocyte is in the center.

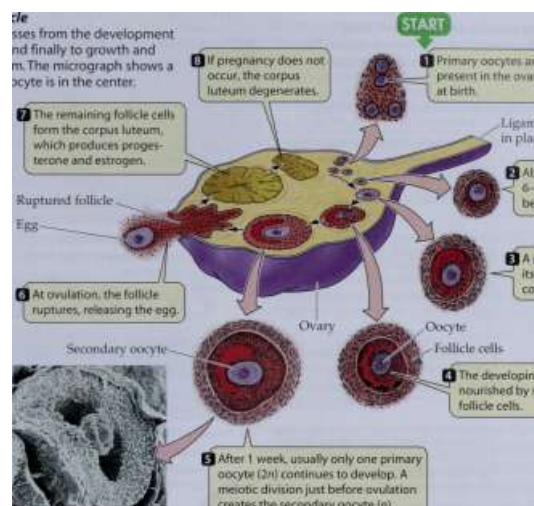


If pregnancy does not occur, the corpus luteum degenerates.

| Primary oocytes are present in the ovary at birth.

I The remaining follicle cells form the corpus luteum, which produces progesterone and estrogen.

Ruptured follicle Egg



Ligament (holds ovary in place in body)

About once a month 6-12 primary oocytes begin to mature.

A primary oocyte and its surrounding cells constitute a follicle.

The developing oocyte is nourished by surrounding follicle cells.

Q After 1 week, usually only one primary oocyte (2n) continues to develop. A meiotic division just before ovulation creates the secondary oocyte (n).

structure, which remains in the ovary, is the corpus luteum (plural corpora lutea). It functions as an endocrine gland, producing estrogen and progesterone for about 2 weeks. It then degenerates unless the egg is fertilized.

The uterine cycle prepares an environment for the fertilized egg

The uterine cycle of human females parallels the ovarian cycle, and consists first of a buildup and then of a breakdown of the endometrium, or uterine lining (Figure 43.13). About 5 days into the ovarian cycle, the endometrium starts to grow in preparation for receiving a blastocyst. The uterus attains its maximum state of preparedness about 5 days after ovulation and remains in that state for another 9 days. If a blastocyst has not arrived by that time, the endometrium begins to break down, slough off, and flow from the body through the vagina—the process of menstruation (from menses, the Latin word for "months").

The uterine cycles of mammals other than humans do not include menstruation; instead, the uterine lining is re-sorbed. In

these species the most obvious correlate of the ovarian cycle is a state of sexual receptivity called estrus around the time of ovulation. When the female comes into estrus, or "heat," she actively solicits male attention and may be aggressive to other females. The human female is unusual among mammals in that she is potentially sexually receptive throughout her ovarian cycle and at all seasons of the year.

Hormones control and coordinate the ovarian and uterine cycles

The ovarian and uterine cycles of human females are coordinated and timed by the same hormones that initiate sexual maturation. Gonadotropins secreted by the anterior pituitary are the central elements of this control. Before puberty (that is, before about 11 years of age), the secretion of gonadotropins is low, and the ovaries are inactive. At puberty, the hypothalamus increases its release of gonadotropin-releasing hormone (GnRH), thus stimulating the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

In response to FSH and LH, ovarian tissue grows and produces estrogen. The rise in estrogen causes the development of female secondary sexual characteristics, including growth of the uterus. Between puberty and menopause, interactions of gonadotropin-releasing hormone, gonadotropins, and sex steroids control the ovarian and uterine cycles.

Menstruation marks the beginning of the uterine and ovarian cycles (see Figure 42.13). A few days before menstruation begins, the anterior pituitary begins to increase its secretion of FSH and LH. In response, some follicles begin to mature in the ovaries, and follicle cells gradually increase production of estrogen. After about a week of growth, usually all but one of these follicles wither away. Occasionally more than one follicle continues to develop, making it possible for the woman to bear fraternal (nonidentical) twins.

744 CHAPTER FORTY-TWO

rn



42.13 The Uterine and Ovarian Cycles

During a woman's uterine and ovarian cycles there are coordinated changes in (a) gonadotropin release by the anterior pituitary, (b) the ovary, (c) the release of female sex steroids, and (d) the uterus. The cycles begin with the onset of menstruation; ovulation is at midcycle.

FSH and LH are under control of GnRH from the hypothalamus and the ovarian hormones estrogen and progesterone.

FSH stimulates the development of follicles; the LH surge causes ovulation and then the development of the corpus luteum.

Ovarian hormones stimulate the development of the endometrium in preparation for pregnancy.

The development of the uterine lining (the endometrium) is controlled by estrogen and progesterone.

(a) Gonadotropins (from anterior pituitary)

Estrogen inhibits

LH and FSH

release

Estrogen stimulates

LH and FSH

release

>~ + > ~4

Estrogen inhibits

LH and FSH

release

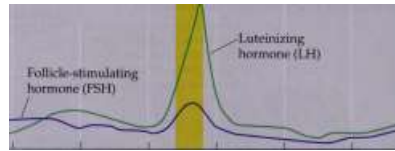
0

10

15

~~Γ~

20



Follicle-stimulating hormone (FSH)

(b) Events in ovary (ovarian cycle)

Developing Oocyte follicle
maturation

© m g G

J I ! L

Corpus luteum Developing oocyte



(c) Ovarian hormones

Estrogen



(d) Events in the endometrium (uterine cycle)

^* Menses

Bleeding and sloughing of uterine lining



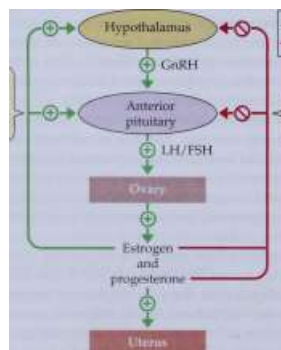
10 15

Day of uterine cycle

20

25

Positive feedback occurs during days 12 through 14.



—©-► = Stimulates —Q+ = Inhibits

Negative feedback occurs throughout most of the cycle.

42.14 Hormones Control the Ovarian and Uterine Cycles

The ovarian and uterine cycles are under a complex series of positive and negative feedback controls involving several hormones.

The follicle that is still growing secretes increasing amounts of estrogen, stimulating the endometrium to grow.

Estrogen exerts negative feedback control on gonadotropin release by the anterior pituitary during the first 12 days of the ovarian cycle. Then, on about day 12, estrogen exerts positive rather than negative feedback control on the pituitary (Figure 42.14). As a result, there is a surge of LH, and a lesser surge of FSH. The LH surge triggers the mature follicle to rupture and release its egg, and it stimulates follicle cells to develop into the corpus luteum and to secrete estrogen and progesterone.

Estrogen and especially progesterone secreted by the corpus luteum following ovulation are crucial to the continued growth and maintenance of the endometrium. In addition, these sex steroids exert negative feedback control on the pituitary, inhibiting gonadotropin release and thus preventing new follicles from beginning to mature.

If the egg is not fertilized, the corpus luteum degenerates on about day 26 of the cycle. Without the production of progesterone by the corpus luteum, the endometrium sloughs off, and menstruation occurs. The decrease in circulating steroids also releases the hypothalamus and pituitary

ANIMAL REPRODUCTION 745

from negative feedback control, so GnRH, FSH, and LH all increase. The increase in these hormones induces the next round of follicle development, and the ovarian cycle begins again.

If the egg is fertilized, and a blastocyst arrives in the uterus and implants itself in the endometrium, a new hormone comes into play. A layer of cells covering the blastocyst begins to secrete human chorionic gonadotropin (hCG). This gonadotropin, a molecular homolog of LH, keeps the corpus luteum functional. Because hCG is present only in the blood of pregnant women, the presence of this hormone is the basis for pregnancy testing.

These tissues derived from the blastocyst also begin to produce estrogen and progesterone, eventually replacing the corpus luteum as the most important source of these sex steroids. Continued high levels of estrogen and progesterone prevent the pituitary from secreting gonadotropins; thus the ovarian cycle ceases for the duration of the pregnancy. The same mechanism is exploited by birth control pills, which contain synthetic hormones resembling estrogen and progesterone that prevent the ovarian cycle (but not the uterine cycle) by exerting negative feedback control on the hypothalamus and pituitary.

Human Sexual Behavior

The organs of the male and female reproductive systems are similar in all eutherians. However, the hormonal and emotional biology of reproductive behavior in humans is more complex. The emotional and social complexities that have evolved to such a tremendous degree in humans have affected our reproductive lives, as have our extensive technological achievements. Here we discuss human sexual responses and technologies for both contraception (birth control) and enhanced fertility. The chapter closes with a discussion of sexually transmitted diseases.

Human sexual responses consist of four phases

The responses of both women and men to sexual stimulation consist of four phases: excitement, plateau, orgasm, and resolution. As sexual excitement begins in a woman, her heart rate and blood pressure rise, muscular tension increases, her breasts swell, and her nipples become erect. Her external genitals, including the sensitive clitoris, swell as they become filled with blood, and the walls of the vagina secrete lubricating fluid that facilitates copulation.

As a woman's sexual excitement increases, she enters the plateau phase. Her blood pressure and heart rate rise further, her breathing becomes rapid, and the clitoris begins to retract—the greater the excitement, the greater the retraction. The sensitivity that once focused in the clitoris spreads over the external genitals, and the clitoris itself becomes even more sensitive. Orgasm may last as long as a few minutes, and, unlike men, some women can experience several orgasms in rapid succession. During the resolution phase, blood drains from the genitals, and body physiology returns to close to normal.

In the male, as in the female, the excitement phase is marked by an increase in blood pressure, heart rate, and muscle tension. The penis fills with blood and becomes hard and erect. In the plateau phase, breathing becomes rapid, the diameter of the glans increases, and a clear lubricating fluid from the bulbourethral glands oozes from the penis. Pressure and friction against the nerve endings in the glans and in the skin along the shaft of the penis eventually trigger orgasm. Massive spasms of the muscles in the genital area and contractions in the accessory reproductive organs result in ejaculation.

Within a few minutes after ejaculation, the penis shrinks to its former size, and body physiology returns to resting conditions. The male sexual response includes a refractory period immediately after orgasm. During this period, which may last 20 minutes or more, a man cannot achieve a full erection or another orgasm, regardless of the intensity of sexual stimulation.

Humans use a variety of technologies to control fertility

People use many methods to control the number of their children and the time between their children's births. The only absolutely sure methods of preventing fertilization and pregnancy are complete abstinence from sexual activity or surgical removal of the gonads. Since those approaches are not acceptable to most people, they turn to a variety of other methods to prevent pregnancy or conception, which therefore are called methods of contraception.

Some methods of contraception are used by the woman, others by the man. They vary from means of blocking gametogenesis to means of blocking development of the embryo. Contraceptive methods vary enormously in their effectiveness and in their acceptability to those who use them. Here we review some of the most common methods and their relative failure rates (Table 42.1).

nontechnological approaches. An approach to contraception that does not involve physical or pharmacological technologies is to separate sperm and egg in time through the rhythm method. The couple avoids sex from day 10 to day 20 of the ovarian cycle, when the woman is most likely to be fertile. The cycle can be tracked by use of a calendar, supplemented by the basal body temperature method, which identifies the day of ovulation on the basis of the observation that a woman's body temperature drops on the day of ovulation and rises sharply on the day after. Changes in the stickiness of the cervical mucus also help identify the day of ovulation.

However, sperm deposited in the female reproductive tract may remain viable for up to 6 days. Similarly, the ovum remains viable for 2 to 3 days after ovulation. These facts, added to individual variation in the timing of ovulation, result in an annual failure rate of between 15 and 35 percent for the rhythm method. In other words, 15 to 35 percent of women using only the rhythm method for 1 year will become pregnant during that time.

746 CHAPTER FORTY-TWO

"T-2 . 1 Methods of Contraception

METHOD

MODE OF ACTION

FAILURE RATE'

Rhythm method Coitus interruptus Condom Diaphragm/jelly

Vaginal jelly or

foam Douche

Birth control pills Vasectomy Tubal ligation

Intrauterine device (IUD)

RU-486

(Unprotected)

Abstinence near time of ovulation Prevents sperm from reaching egg Prevents sperm from entering vagina Prevents sperm from entering uterus;

kills sperm Kills sperm; blocks sperm movement

Supposedly flushes sperm from vagina Prevent ovulation Prevents release of sperm Prevents egg from entering uterus Prevents implantation of fertilized egg

Prevents development of fertilized egg (No form of birth control)

' Number of pregnancies per 100 women per year

Another approach is to try to separate sperm and egg in space through coitus interruptus —withdrawal of the penis before ejaculation. The annual failure rate of this method may be as high as 40 percent.

barrier methods. Techniques for placing a physical barrier between egg and sperm have been used for centuries. The condom is a sheath made of an impermeable material such as latex that can be fitted over the erect penis. A condom traps the semen so that sperm do not enter the vagina. Condoms also help prevent the spread of sexually transmitted diseases such as AIDS, syphilis, and gonorrhea. In theory, the use of a condom can be highly effective, with a failure rate near zero; in practice, the annual failure rate is about 15 percent, because of faulty technique. There is also a female condom, which can be used by the woman to create an impermeable lining of the vagina.

The diaphragm is a dome-shaped piece of rubber with a firm rim that fits over the woman's cervix and thus blocks sperm from entering the uterus. Smaller than the diaphragm is the cervical cap, which fits snugly just over the tip of the cervix. Both the diaphragm and the cervical cap are treated first with jelly or cream containing a spermicide —a chemical that kills or incapacitates sperm— and then inserted through the vagina before sexual intercourse. Annual failure rates are about the same as for condoms—about 15 percent.

Spermicidal foams, jellies, and creams can be used alone by placing them in the vagina with special applicators. Used in this way, they have an annual failure rate of 25 percent or more. Douching (flushing the vagina with liquid) after intercourse, in spite of popular belief, is almost useless as a method of birth control. Remember that sperm can reach the upper regions of the oviducts within 10 minutes after ejaculation.

15-35 10-40 3-20 3-25

The effectiveness of barrier methods can be greatly improved if different ones are used in combination. For example, if the man uses a condom and the woman a diaphragm, the failure rate is extremely low.

preventing ovulation. The widely used oral contraceptives, or birth control pills, work by preventing ovulation. Their mechanisms of action take advantage of the roles of estrogens and progesterone as negative feedback signals that work on both the hypothalamus and the pituitary to inhibit gonadotropin release. The most common pills contain low doses of synthetic estrogens and progesterones (progestins). By keeping the circulating levels of gonadotropins low, the birth control pill interferes with the maturation of follicles and ova. The ovarian cycle (but not the uterine cycle) is suspended. The negative side effects of oral contraceptives have been the topic of extensive discussion. These side effects include increased risk of blood clot formation, heart attack, stroke, and breast cancer. However, these side effects are associated mostly with pills containing higher hormone concentrations than are used in modern pills. For pills in use today, the risk of these side effects is low, except for women over 35 years old who smoke, for whom the risk is significantly greater. Risk of death from using "the pill" is less than the risk associated with a full-term pregnancy. The pill is the most effective method of contraception other than sterilization or perhaps combined barrier methods. Oral contraceptives have an annual failure rate of less than 1 percent.

The "mini-pill" is an oral contraceptive that contains very low doses of progestins. Although it may interfere with the normal maturation and release of ova, its principal mode of action is to alter the environment of the female reproductive tract so that it is not hospitable to sperm. Cervical mucus normally becomes watery at the time of ovulation, but low levels of progestin keep the mucus thick and sticky so that it blocks the passage of sperm.

Long-lasting injectable or implantable steroids are also used to block ovulation. DepoProvera is an injectable progestin that blocks normal pituitary function for several months. Another device, called Norplant, consists of thin, flexible tubes filled with progestin. Several of these tubes are inserted under the skin, where they continue to release progestin slowly for years.

preventing implantation. A highly effective method of contraception (with a failure rate varying from 1 percent to about 7 percent) is the intrauterine device, or IUD. The IUD is a small piece of plastic or copper that is inserted in the uterus. The IUD probably works by preventing implantation of the fertilized egg.

Another way of interfering with implantation is through the use of "morning-after pills/" which deliver high doses of steroids, primarily estrogens. By acting in several ways on the oviduct and the uterine lining, this treatment prevents implantation. Morning-after pills can be effective up to several days after intercourse.

A recent addition to birth control technology is a drug, RU-486, developed in France. RU-486 is not a contraceptive pill, but a contragestatiotml pill. It is a progesterone-like molecule that blocks progesterone receptors. It therefore interferes with the normal action of progesterone produced by the corpus luteum, which is necessary for the maintenance of the uterine lining in early pregnancy. If RU-486 is administered as a "morning-after pill", it prevents implantation. However, RU-486 can be effective even if taken at the time of the first missed menses after fertilization, after implantation has been initiated. After a few days of treatment with RU-486, the endometrium regresses and sloughs off along with the embryo, which is in very early stages of development.

sterilization. One virtually foolproof method of contraception is sterilization of either the man or the woman. Male sterilization by vasectomy is a simple operation that can be performed under a local anesthetic in a doctor's office (Figure 42.15a). After this minor surgery, the semen no longer contains sperm. Sperm production continues, but since the sperm cannot move out of the testes, they are destroyed by macrophages. Vasectomy does not affect a man's hormone levels or his sexual responses, and even the amount of semen he ejaculates is unchanged, because the sperm constitute so little of its volume.

In female sterilization, the aim is to prevent the egg from traveling to the uterus and to block sperm from reaching the egg. The most common method is tubal ligation —cutting and tying the oviducts (Figure 42.15b). Alternatively, the oviducts may be burned (cauterized) to seal them off, using a surgical technique called endoscopy. As in the male, these procedures do not alter reproductive hormones or sexual responses.

abortion. Once a fertilized egg has successfully implanted itself in the uterus, the termination of a pregnancy is called an abortion. A spontaneous abortion is the medical term for what is usually called a miscarriage. Miscarriages are common early in pregnancy; most of them occur because of an abnormality in the fetus or in the process of implantation. Abortions that are not spontaneous, but are the result of medical intervention, may be done either for therapeutic or for contraceptive purposes. A therapeutic abortion may be necessary to protect the health of the mother, or it may be performed because prenatal testing (which will be discussed in the following chapter) reveals that the fetus has a severe defect.

When performed in the first third of a pregnancy, a medical abortion carries less risk than a full-term pregnancy. The method is to dilate the cervix and then remove the

(a) Vasectomy

A short piece of vas deferens is cut out...

...and the ends are tied off.

Vas deferens



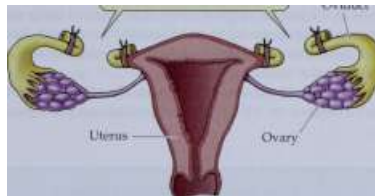
Testis Scrotum



(b) Tubal ligation

The oviducts are cut and the ends are folded over and tied off.

Oviduct



42.75 Sterilization Techniques

(a) Vasectomy is the technique for male sterilization. (b) Tubal ligation is the sterilization procedure performed on human females.

fetus and the endometrium by physical means. After the first 12 weeks of pregnancy, the risk associated with a medical abortion rises substantially.

controlling male fertility. You may ask why all the chemical approaches to controlling fertility apply to females and none have been devised to block male fertility. The control of male fertility is a difficult problem. First, since the production of sperm is a continuous, rather than a cyclical, event, it is not possible to block a particular step in a cyclical process. The ovarian cycle is vulnerable to manipulation because certain events must happen at certain times for ovulation and implantation to occur. Second, the suppression of spermatogenesis must be total to be effective, since it takes only a single sperm to fertilize an egg, and normally millions are produced. Such suppression requires powerful and continuous chemical intervention with associated side effects.

Reproductive technologies help solve problems of infertility

There are many reasons why a man and woman may not be able to have children. The man's rate of sperm production may be low, or his sperm may lack motility. The mucus in the woman's reproductive tract may be thick and not conducive to sperm moving up to the oviducts. Structural

748 CHAPTER FORTY-TWO

problems may also exist, such as a woman's oviducts being blocked by scar tissue or by endometriosis, a proliferation of endometrial cells outside of the uterus. In some cases, treatment with powerful chemicals to cure cancer damages the ability of the gonads to produce gametes.

Even a couple who are fully fertile and who want children may not want to take the risk of the natural process of fertilization if one or both parents are carriers for a genetic disease. A number of reproductive technologies have been developed to overcome these and other barriers to childbearing.

The oldest and simplest reproductive technology is artificial insemination, which involves placing sperm in the appropriate place in the female's reproductive tract for fertilization to occur. This technique is useful if the male's sperm count is low, if the sperm lack motility, or if problems in the female's reproductive tract prevent the normal movement of sperm up to and through the oviducts. Artificial insemination is used widely in the production of domesticated animals such as cattle.

More recent advances, called assisted reproductive technologies, or ART's, involve procedures that remove

unfertilized eggs from the ovary, combine them with sperm outside of the body, and then place fertilized eggs or egg/sperm

mixtures in the appropriate location in the female's reproductive tract for development to take place.

The first successful ART was in vitro fertilization (IVF). In IVF, the mother is treated with hormones that stimulate many follicles in her ovaries to mature. Eggs are harvested from these follicles, and sperm are collected from the father. Eggs and sperm are combined in a culture medium outside the body (in vitro), where fertilization takes place. The resulting embryos can then be injected into the mother's uterus in the blastocyst stage or kept frozen for implantation later. The first "test tube baby" resulting from IVF was born in 1978. Since that time, thousands of babies have been produced by this ART. IVF is useful when the woman's oviducts are blocked. It has a success rate of 20 to 25 percent.

A technique called gamete intrafallopian transfer (GIFT) can be used when only the entrance to the oviducts from the ovaries, or the upper segment of the oviducts, is blocked. In this procedure, harvested eggs and sperm are

<tfZ Some Sexually Transmitted Diseases

DISEASE

INCIDENCE IN UNITED STATES

SYMPTOMS

Syphilis

Gonorrhea

Chlamydia

Genital herpes

80,000 new cases/yr

800,000 new cases/yr

>4,000,000 new cases/yr

500,000 new cases/yr

Primary stage (weeks): skin lesion (chancre) at site of infection Secondary stage (months): skin rash and flu-like symptoms, may be

followed by a latent period Tertiary stage (years): deterioration of the cardiovascular and central

nervous systems

Pus-filled discharge from penis or vagina; burning urination. Infection can also start in throat or rectum

Symptoms similar to gonorrhea, although often there are no obvious symptoms. Can result in pelvic inflammatory disease in females (see below)

Small blisters that can cause itching or burning sensations are accompanied by inflammation and by secondary infections

Genital warts

10% of adults infected

Small growths on genital tissues. Increases risk of cervical cancer in women

Hepatitis B

5-20% of population

Fatigue, fever, nausea, loss of appetite, jaundice, abdominal pain, muscle and joint pain. Can lead to destruction of liver or liver cancer

Pelvic inflammatory 1,000,000 new cases/yr disease (females only)

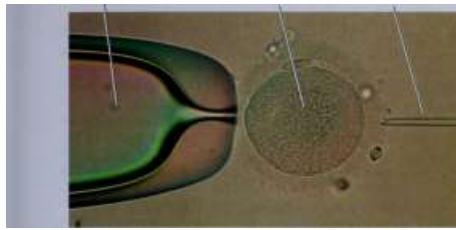
AIDS

Fever and abdominal pain. Frequently results in sterility Approximately 900,000 cases" Failure of the immune system (see Chapter 19)

'AIDS is widespread in other parts of the world, most notably in the southern part of the African continent, where some 9 million people are infected. The virus is also spreading rapidly in India and Southeast Asia.

Holding pipette

Egg Pipette injecting sperm



42.16 Intracytoplasmic Sperm Injection

In this procedure, sperm are injected directly into a mature egg cell. The fertilized egg can then be placed back in the female reproductive tract.

injected directly into the upper regions of the oviduct, where fertilization takes place, and the blastocyst enters the uterus via the normal route. GIFT has a success rate of about 30 percent.

ANIMAL REPRODUCTION 749

A major cause of failure of IVF and GIFT is the failure of sperm to gain access to the plasma membranes of eggs. To solve this problem, methods have been developed to inject a sperm cell directly into the cytoplasm of an egg. In intracytoplasmic sperm injection (ICSI), a harvested egg is held in place by suction applied to a polished glass pipette. A slender, sharp pipette is then used to penetrate the egg and inject a sperm (Figure 42.16). This ART was successful for the first time in 1992; now thousands of these procedures are performed in U.S. clinics each year, with a success rate of about 25 percent.

IVF coupled with sensitive techniques of genetic analysis, can eliminate the risk that parents who are carriers for genetic diseases will produce affected children. With in vitro fertilization, it is possible to take a cell from the blastocyst at the 4- or 8-cell stage without damaging its developmental potential. The sampled cell can then be subjected to molecular techniques to determine whether it carries the harmful gene. This information guarantees that only embryos that will not develop the genetic disease are implanted in the mother's uterus.

CAUSE

MODE OF TRANSMISSION

CURE/TREATMENT

Spirochete bacterium (*Treponema pallidum*) that penetrates mucosal membranes and abraded skin

Intimate sexual contact (even kissing)

Antibiotics

Bacterium (*Neisseria gonorrhoeae*)

Bacterium (*Chlamydia trachomatis*)

Communicated across mucous membranes

Communicated across mucous membranes

Antibiotics (but antibiotic-resistant strains have arisen)

Antibiotics

Herpes simplex virus Human papillomavirus Virus

A variety of bacteria that migrate to the uterus and fallopian tubes

HTV (see Chapter 13)

Communicated by contact with infected surfaces, which can be mucous membranes or skin

Communicated across mucous membranes through sexual contact

Sexual contact or blood transfusions

Sexual intercourse

No cure. Symptoms can be alleviated. Antiviral drugs may lessen subsequent outbreaks

No cure for the virus. Warts can be removed surgically or by burning, freezing, or chemical treatment

No cure. Symptoms can be treated. A vaccine is available that can protect only if given before infection occurs

Antibiotics

The virus enters the bloodstream via cuts or abrasions, including minute ones in the genitalia. Spread primarily by intimate sexual contact, but can also be transmitted via contaminated needles

No cure. Treatments with a variety of medications can slow the course of the infection

750 CHAPTER FORTY-TWO

Sexual behavior transmits many disease organisms

Disease-causing organisms are parasites, and have a very limited ability to survive outside a host organism. Therefore, getting from host to host is a major evolutionary challenge for disease-causing organisms. One of the most intimate types of contact that hosts can have is copulation. It is not surprising, then, that many parasitic organisms have evolved to depend on sexual contact between their hosts as their means of transmission. These organisms are the causes of sexually transmitted diseases (commonly referred to as STD's), and they include viruses, bacteria, yeasts, and protozoans.

STD's have been with humans since ancient times, and they are one of the most serious public health problems today. Over 10 million new cases of STD's occur each year in the United States, and about two-thirds of these cases occur in people between the ages of 15 and 30. About half of the youth in this country will contract an STD before the age of 25.

A summary of the most common STD's is presented in Table 42.2 on the preceding page. The highly prevalent bacterially transmitted diseases chlamydia and gonorrhea are generally not fatal, but when untreated are a major cause of infertility and painful inflammatory diseases. Syphilis is transmitted by a spirochete and is fatal in about half of untreated cases. It is believed that syphilis was brought to the Old World from the New World by the crew of Christopher Columbus, who himself died of advanced syphilis 16 years after his first voyage to North America. AIDS, transmitted by a virus, is an STD of recent origin and currently has a high rate of mortality. The only device that is effective against the transmission of STD's is the condom.

Chapter Summary Asexual Reproduction

► Sexual reproduction is almost universal in animals, but some animals can reproduce asexually, producing offspring that are genetically identical to their parent and to one another. A disadvantage of asexual reproduction is that no genetic diversity is produced.

► Means of asexual reproduction include budding, regeneration, and parthenogenesis. Review Figures 42.1, 42.2

Sexual Reproduction

► Sexual reproduction consists of three basic steps: gametogenesis, mating, and fertilization. Gametogenesis and fertilization are similar in all animals, but mating includes a great variety of anatomical, physiological, and behavioral adaptations.

► In sexually reproducing species, genetic diversity is created by the recombination of genes during gametogenesis and by the independent assortment of chromosomes. Mating and fertilization also contribute to genetic diversity.

► Gametogenesis occurs in testes and ovaries. In spermatogenesis (the production of sperm) and oogenesis (the production of eggs), the primary germ cells proliferate mitotically, undergo meiosis, and mature into gametes. Review Figure 42.4

► Spermatogonia continue to proliferate by mitosis throughout the life span of the male. Each primary spermatocyte can produce four haploid sperm through the two divisions of meiosis. Review Figure 42.4a

► Primary oocytes immediately enter prophase of the first meiotic division, and in many species, including humans, their development is arrested at this point. Each oogonium produces only one egg through meiosis. Review Figure 42.4b

► Hermaphroditic species have both male and female reproductive systems in the same individual, either sequentially or simultaneously.

► Fertilization can occur externally, which is common in aquatic species, or internally, which is common in terrestrial species. Internal fertilization usually involves copulation.

► Internal fertilization is necessary for nonaquatic species. The shelled egg is an important adaptation to the desiccating terrestrial environment, but it must be fertilized before the shell forms. All mammals except monotremes retain the embryo internally and have done away with shelled eggs.

► Animals can be classified as oviparous or viviparous depending on whether the early stages of development occur outside or inside the mother's body.

The Human Reproductive System

► Males produce and deliver semen into the female reproductive tract. Semen consists of sperm suspended in a fluid that nourishes them and facilitates fertilization.

► Sperm are produced in the seminiferous tubules of the testes, mature in the epididymis, and are delivered to the urethra through the vasa deferentia. Other components of semen are produced in the seminal vesicles, prostate gland, and bulbourethral glands. All components of the semen join in the urethra at the base of the penis and are ejaculated through the erect penis by muscle contractions at the culmination of copulation. Review Figures 42.8, 42.9

► Spermatogenesis depends on testosterone secreted by Leydig cells in the testes, which are under control of luteinizing hormone from the pituitary. Spermatogenesis is also controlled by follicle-stimulating hormone from the pituitary. Hypothalamic gonadotropin-releasing hormone controls pituitary secretion of LH and FSH. The production of these hormones by the hypothalamus and pituitary is controlled by negative feedback from testosterone and another hormone produced by the testes, inhibin. Review Figure 42.10

► Eggs (ova) mature in the female's ovaries and are released into the oviducts, which deliver the eggs to the uterus. Sperm deposited in the vagina during copulation move up through the cervix into the uterus, some continuing up through the oviducts. Review Figure 42.11

► Fertilization occurs in the upper regions of the oviducts. The zygote becomes a blastocyst through repeated cell divisions as it passes down the oviduct. Upon arrival in the uterus, the blastocyst implants itself in the endometrium, where a placenta forms and the embryo develops.

► The maturation and release of ova constitute an ovarian cycle under the control of the anterior pituitary hormones FSH and LH. In humans, this cycle takes about 28 days. Review Figures 42.12, 42.13

► The uterus also undergoes a cycle that prepares it for receipt of a blastocyst. If no blastocyst is implanted, the lining of the uterus deteriorates and sloughs off, which is the process of menstruation. Review Figure 42.13

► Both the ovarian and the uterine cycles are under the control of hypothalamic and pituitary hormones, which in turn are under the feedback control of estrogen and progesterone. Review Figure 42.14

ANIMAL REPRODUCTION 751

Human Sexual Behavior

► Human sexual responses consist of four phases: excitement, plateau, orgasm, and resolution. In addition, males have a refractory period during which renewed excitement is not possible.

► Methods to prevent pregnancy include abstention from copulation or the use of technologies that decrease the probability of fertilization. Review Table 42.1

► Barrier methods of contraception, such as condoms, diaphragms, and spermicidal substances, block the passage of sperm in the female reproductive tract or weaken and kill them.

► Methods to prevent ovulation, such as birth control pills and other hormonal treatments, interfere with the ovarian cycle so that mature, fertile ova are not produced and released.

► Males and females can be sterilized by surgical blockage of the vasa deferentia (vasectomy) or oviducts (tubal ligation). Review Figure 42.15

► Methods to prevent implantation of a blastocyst include intrauterine devices, excess doses of steroids, and a progesterone receptor blocker. After implantation, the termination of a pregnancy is called an abortion.

► Assisted reproductive technologies have been developed to increase fertility. ARTs include in vitro fertilization and gamete intrafallopian transfer.

► Sexually transmitted diseases result from the transmission of disease-causing organisms through sexual behavior. Many STD's are curable if treated early, but can have serious long-term consequences if not treated. Review Table 42.2

For Discussion

1. In the very deep ocean, there are species of fish in which the male is very much smaller than the female and actually lives attached to her body. In terms of the selective pressures that operate on sexual and asexual reproduction and in terms of the deep-sea environment, what factors do you think resulted in the evolution of this extreme sexual dimorphism?
2. What are two main differences between the immediate products of the first and second meiotic division in spermatogenesis and oogenesis? Why do these differences exist?
3. At the beginning of each ovarian cycle in humans, about six follicles begin to develop in response to rising levels of FSH, but after a week, only one follicle continues to develop, and the others wither away. Given the facts that follicles produce estrogen, estrogen stimulates follicle cells to produce FSH receptors, and estrogen exerts negative feedback on FSH production in the pituitary, how can you explain how one follicle gets "selected" to grow?
4. Compare the actions of LH and FSH in the ovaries and testes.
5. Ovarian and uterine events in the month following ovulation differ depending on whether fertilization occurs. Describe the differences and explain their hormonal controls.



The whale blows its nose out the top of its head—as in "thar she blows," the infamous whalers' cry. The spout coming out of the blowhole is the whale's exhalation coming out of its nasal passages. It is convenient for a marine mammal to be able to breathe out of the top of its head. Not much of its body has to come out of the water for it to breathe, and it can continue moving forward through the water while breathing. But in most mammals, the nose is on the front of the head. How did the whale's nose happen to get to the top of its head? This is an evolutionary question, but the answer is to be found in embryological development.

The vertebrate body varies enormously among species in form and function, yet its basic structural design is highly conserved. For example, the whale flipper, the bat wing, and the human arm all have the same bones. However, during development these bones assume different shapes and dimensions to adapt the forelimbs to their various functions: swimming, flying, and tool use.

Similarly, vertebrates all have the same bones in their heads, but through development, these bones grow differentially, and therefore the skull takes on different shapes. In both the whale and the human, the nasal passages are in the nasal bone, which in both is just above the bones of the upper jaw. In the human, that places the nasal bone just above the jaw on the front of the face. During development of the whale skull, however, the bones of the upper jaw grow enormously relative to the other bones of the skull, and project far forward to form the cavernous mouth. As a result of this differential forward growth of the jaw bone, the nasal bone ends up on the top of the skull rather than on the front. The answer to why the whale's nose is on the top of its head and how forelimbs become flippers is found in the processes of development that form and shape the components of the basic vertebrate body plan.

Starting from the organization of the fertilized egg, signaling cascades unfold

Thar She Blows!

The nasal passages of the whale *Orcinus orca* are on top of its head because of the extreme growth of its jaw bones during development.

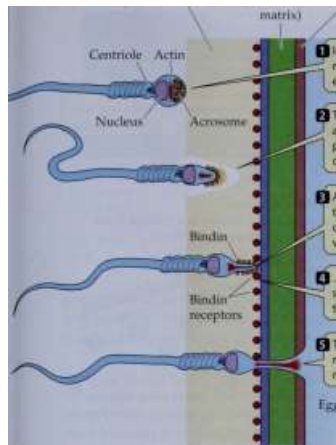
that control the building of the body. Typically, molecules acting as transcription factors influence the temporal and spatial expression of still other genes that control the growth and differentiation of cells. Interactions between cells set up ever more complex signaling relationships that result in the structural and functional differences between species. The chemical and genetic nature of these signals is remarkably similar over widely different species, but the exciting story of development is how these signals are used in different patterns and combinations to produce what we see as different species.

In this chapter we trace the early stages of embryonic development. We begin with the events of fertilization, which can be taken as the starting point for the development of the organism. From there we see how the zygote is converted by rapid cell divisions into a mass of cells. These cells then change position relative to each other, establishing new contacts between different groups of cells. These contacts initiate sequences of changes in cell growth, cell movements, and cell differentiation from which emerge the overall body plan, the various tissues, and the rudimentary organs of the adult.

To appreciate both the diversity and the similarity in the development of different animals, we discuss these early developmental steps in four organisms studied extensively by developmental biologists: sea urchins (invertebrates) and frogs, chicks, and humans (all vertebrates).



Jelly layer Vitelline envelope Egg plasma (extracellular coat) (extracellular membrane matrix)



In the sperm head the nucleus is capped by an enzyme-filled acrosome.

These enzymes digest a path through the protective jelly coat of the egg.

Q An acrosomal process then forms and makes contact with the vitelline envelope...

...which has species-specific receptors for the protein bindin.

The acrosomal process membrane and egg membrane fuse.



Egg cytoplasm

43.1 The Acrosomal Reaction

The acrosomal reaction allows a sea urchin sperm to recognize an egg of the same species and pass through its protective layers.

Fertilization: Interactions of Sperm and Egg

Development in sexually reproducing animals begins with fertilization, the union of sperm and egg to create a single cell: a diploid zygote. Fertilization requires a complex series of events:

- ▶ The sperm and the egg must recognize each other.
- ▶ The sperm must be activated so that it is capable of gaining access to the plasma membrane of the egg.
- ▶ The plasma membranes of the sperm and the egg must fuse.
- ▶ The egg must block entry by additional sperm.
- ▶ In mammals, the egg nucleus must complete its final meiotic division.
- ▶ The egg and sperm nuclei must fuse to create the diploid nucleus of the zygote.

We will look at each of these steps in turn.

Recognition molecules assure specificity in sperm-egg interactions

Specific recognition molecules mediate interactions between sperm and eggs. These molecules assure that the activities of the sperm are directed toward eggs and not other cells, and they help prevent eggs from being fertilized by sperm from the wrong species. The latter function is particularly important in species that engage in external fertilization. The sea urchin is such a species, and its mechanisms of fertilization have been well studied.

ANIMAL DEVELOPMENT 753

The plasma membrane of the sea urchin egg is protected by a proteinaceous vitelline envelope, and surrounding that is a jelly coat. The sperm must get through these two protective layers before it can fuse with the plasma membrane of the egg. To accomplish this, the sperm has a membrane-enclosed structure called an acrosome containing enzymes and other proteins. The acrosome is located at the front end of the sperm head and covers the nucleus.

When the sperm makes contact with an egg of its own species, substances in the jelly coat trigger an acrosomal reaction, which begins with the release of acrosomal enzymes that digest the jelly coat. Next, an acrosomal process extends out of the head of the sperm. The acrosomal process forms from globular actin behind the acrosome, which polymerizes when the acrosomal membrane breaks down (Figure 43.1).

The acrosomal process extends through the jelly coat to make contact with the vitelline envelope. Herein lies another species

recognition mechanism: The acrosomal process is coated with a membrane-bound protein called bindin. Different species have different bindin molecules. The egg plasma membrane has species-specific bindin receptors that extend through the vitelline envelope. The reaction of acrosomal bindin with these receptors stimulates the egg membrane to form a fertilization cone that engulfs the sperm head, bringing it into the egg cytoplasm.

In animals that practice internal fertilization, mating behaviors help guarantee species specificity, but egg-sperm recognition mechanisms still exist. The mammalian egg is surrounded by a thick layer called the cumulus, which consists of follicle cells in a gelatinous matrix (Figure 43.2). Beneath the cumulus is a glycoprotein envelope called the zona pellucida, which is functionally similar to the vitelline envelope of sea urchin eggs. When sperm are first de-

Cumulus

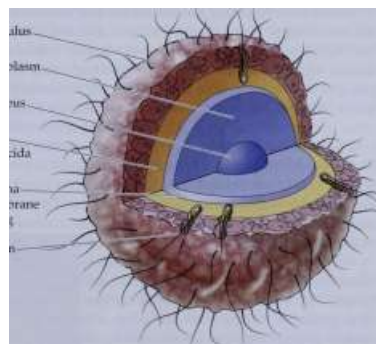
Cytoplasm

Nucleus

Zona pellucida

Plasma membrane of egg

Sperm



43.2 Barriers to Mammalian Sperm

This human egg is protected by the cumulus and zona pellucida, both of which a sperm must penetrate to fertilize the egg.

754 CHAPTER FORTY-THREE

posited in the \ aguaa, they are not capable of mounting an acrosomal reaction, but after being in the female reproductive tract for a time, the sperm undergo capacitation. A capacitated sperm is capable of interacting with an egg and its barriers, probably because certain critical proteins on its surface have been altered to make them reactive.

Mammalian sperm have enzymes on their surface that help them digest a path through the cumulus. When they make contact with the zona pellucida, a species-specific glycoprotein in the zona binds to recognition molecules on the head of the sperm. This binding triggers the acrosomal reaction, releasing acrosomal enzymes that digest a path through the zona. Other egg plasma membrane proteins then bind to adhesive proteins on the sperm, facilitating the fusion of sperm and egg plasma membranes.

The importance of the zona pellucida and its binding molecules in protection against heterospecific interactions between sperm and eggs was revealed in experiments in which the zona was stripped from eggs before they were exposed to sperm in a culture dish. In these experiments, it was possible for hamster sperm to fertilize human eggs, creating a hamster-human hybrid zygote. The hybrid zygote did not survive its first cell division because of chromosomal incompatibilities, but the experiment demonstrated that the mammalian species recognition mechanism resides in the zona.

Sperm entry triggers blocks to polyspermy and activates the egg

The fusion of the sperm and egg plasma membranes and the entry of the sperm into the egg initiate a programmed sequence of events. The first responses to sperm entry are blocks to polyspermy —that is, mechanisms that prevent more than one sperm from entering the egg. If more than one sperm enters the egg, the resulting embryo probably will not survive.

Blocks to polyspermy have been studied intensively in sea urchin eggs, which can be fertilized in a dish of seawater. Within a tenth of a second after a sperm enters a sea urchin egg, there is an influx of sodium ions, which changes the electric potential across the egg's plasma membrane. This fast block to polyspermy prevents the fusion of other sperm with the egg plasma membrane (Figure 43.3).

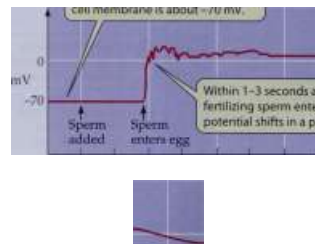
The slow block to polyspermy takes about a minute (Figure 43.4). Before fertilization, the vitelline envelope is bonded to the plasma membrane. Just under the plasma membrane are cortical granules consisting of enzymes and other proteins. The sea urchin egg, like all animal cells, contains calcium that is sequestered in endoplasmic reticulum.

When a sperm enters, the sea urchin egg releases calcium from its endoplasmic reticulum into its own cytoplasm. This increase in cytoplasmic calcium causes the cortical granules to fuse with the plasma membrane. The cortical granule enzymes are released by exocytosis, breaking the bonds between the vitelline envelope and the plasma membrane. Water then flows

into the space between the vitelline envelope and the plasma membrane, raising the vitelline envelope to form the fertilization envelope.

f'. ^

Before the addition of sperm, the potential difference across the egg cell membrane is about -70 mV.



Within 1-3 seconds after the fertilizing sperm enters the egg, the potential shifts in a positive direction.

enters egg

J I i i

0

20

60

80

40 Time (s)

43.3 The Fast Block to Polyspermy

The fast block to polyspermy in sea urchins is a change in the electric potential across the egg plasma membrane.

lope. The enzymes also degrade unused sperm-binding molecules at the surface of the fertilization envelope and cause it to harden, preventing the passage of additional sperm.

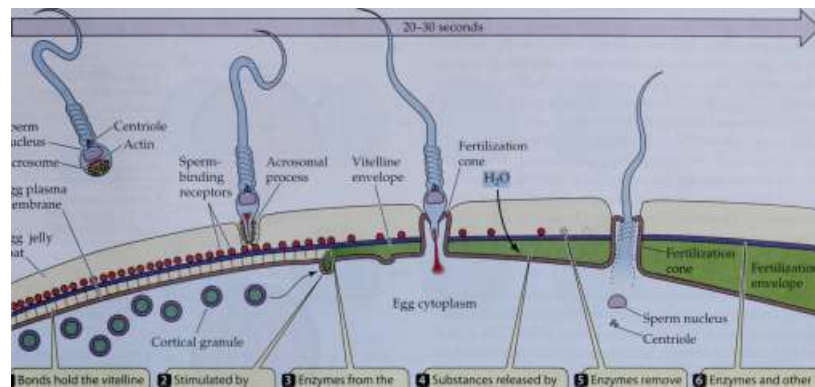
In mammals, sperm entry does not seem to cause a rapid change in membrane potential, but it does trigger the phosphatidylinositol (PTI) signaling system (see Figure 15.13), resulting in several events. Calcium is released from the endoplasmic reticulum, and the egg's metabolism is activated. The pH of the cytoplasm increases, oxygen consumption rises, and protein synthesis increases.

In mammals, the nuclei of the sperm and the egg do not fuse until about 12 hours after the sperm nucleus is taken into the egg cytoplasm because the nucleus of the egg must still complete its second meiotic division (see Figure 42Ab). Sea urchin eggs have already completed the second meiotic division at the time of sperm entry, and the nuclei fuse within an hour to create the zygote.

The sperm and the egg make different contributions to the zygote

Nearly all of the cytoplasm of the zygote comes from the egg. This cytoplasm is well stocked with nutrients, ribosomes, and a variety of molecules, including mRNAs. Moreover, because the sperm mitochondria degenerate, all of the mitochondria in the zygote come from the egg. In addition to its haploid nucleus, the sperm makes one other important contribution to the zygote in some species: a centriole. This centriole becomes the centrosome of the zygote, which produces the mitotic spindles for subsequent cell divisions.

For a long time, it was assumed that the one thing that sperm and egg contributed equally to the zygote was their haploid nuclei. However, we now know that even though they are equivalent in terms of genetic material, mammalian sperm and eggs are not equivalent in terms of their roles in development. In the laboratory, it is possible to construct zygotes in which both haploid nuclei come from the mother or both come from the father. In neither case does development progress normally. Apparently, in mammals at least, certain genes involved in development are active only if they come from a sperm and others are active only if they come from an egg—a phenomenon that has been termed genomic imprinting.



Sperm nucleus

Acrosome

Egg plasma membrane

Egg jelly coat

| Bonds hold the vitelline envelope to the plasma membrane.

^ Stimulated by the release of intracellular Ca^{2+} , cortical granules fuse with plasma membrane.

§) Enzymes from the cortical granules dissolve bonds between the vitelline envelope and the plasma membrane

Q Substances released by the cortical granules absorb H_2O and swell, raising the vitelline envelope away from the plasma membrane.

Enzymes remove

sperm-binding

receptors.

rTl



43.4 The Slow Block to Polyspermy

Enzymes from the sea urchin egg's cortical granules trigger the slow block to polyspermy.

Fertilization causes rearrangements of egg cytoplasm

The entry of the sperm into the egg stimulates changes and rearrangements of the cytoplasm that establish the symmetry and the body axes of the embryo. The nutrients and molecules in the cytoplasm of the zygote are not homogeneously distributed, and are not divided equally among all daughter cells when cell divisions begin. This unequal division of cytoplasmic factors sets the stage for the unfolding series of signals that orchestrates the sequential steps of development: determination, differentiation, and morphogenesis (see Chapter 16).

The rearrangements of egg cytoplasm in some species of frogs can be easily observed because of pigments in the egg

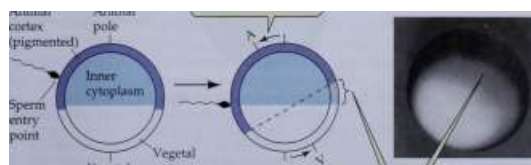
Animal

cortex

(pigmented)

Animal pole

The cortical cytoplasm rotates relative to the inner cytoplasm.



Vegetal pole

Vegetal

cortex

(unpigmented)

The gray crescent is created by the rotation.

Q Enzymes and other substances released from cortical granules also harden the vitelline envelope to form a fertilization envelope, releasing sperm bound to it.

cytoplasm. The nutrient molecules in an unfertilized frog egg are dense, and are therefore concentrated by gravity in the lower half of the egg, which is called the vegetal hemisphere. The haploid nucleus of the egg is located at the opposite end of the egg, in the animal hemisphere. The outermost (cortical) cytoplasm of the animal hemisphere is heavily pigmented, and the underlying cytoplasm has more diffuse pigmentation. The vegetal hemisphere is not pigmented.

Sperm always enter the frog egg in the animal hemisphere. When this occurs, the cortical cytoplasm rotates toward the site of sperm entry. This rotation exposes a band of diffusely pigmented cytoplasm on the side of the egg opposite the site of sperm entry. This band, called the gray crescent, will be the site of important developmental events (Figure 43.5).

The cytoplasmic rearrangements that create the gray crescent bring different regions of cytoplasm into contact on opposite sides of the egg. Therefore, bilateral symmetry is imposed on what was a radially symmetrical egg. Instead of just the up-down difference of the animal and vegetal hemispheres, the movement of the cytoplasm sets the stage for the creation

43.5 The Gray Crescent

Rearrangements of the cytoplasm of frog eggs after fertilization create the gray crescent.

756 CHAPTER FORTY-THREE

of the anterior-posterior and left-right axes. In the frog, the site of sperm entry will become the ventral (belly) region, and the gray crescent will become the dorsal (back) region of the embryo. Since the gray crescent also marks the posterior end of the embryo, these relationships specify the anterior-posterior and left-right axes as well.

The molecular mechanism of this critical first step in embryo formation is beginning to be understood. The sperm centriole rearranges the microtubules in the vegetal pole cytoplasm into a parallel array that presumably guides the movement of the cortical cytoplasm. Organelles and certain proteins from the vegetal hemisphere move to the gray crescent region even faster than the cortical cytoplasm rotates.

As a result of these movements of cytoplasm, proteins, and organelles, changes in the distribution of critical developmental signals occur. A protein kinase called GSK-3 and a protein called β -catenin become unevenly distributed in the cytoplasm of the frog zygote. β -catenin is produced from maternal mRNA found throughout the cytoplasm of the egg. Once the zygote becomes a multicellular embryo, β -catenin will act as a transcription factor in the nuclei of those cells where it is present. GSK-3, which is also present throughout the egg cytoplasm, causes the degradation of β -catenin. However, inhibitors of GSK-3 migrate along the vegetal microtubules and prevent GSK-3 from degrading β -catenin (Figure 43.6). As a result, there is a higher concentration of β -catenin on what will become the dorsal side of the embryo.

Evidence supports the hypothesis that β -catenin is a key player in the cell-cell signaling cascade that begins the formation of the embryo in the region of the gray crescent. But before there can be cell-cell signaling, there must be multiple cells.

Cleavage: Repackaging the Cytoplasm

At some point after fertilization is complete (the exact timing differs among species), a rapid series of cell divisions called cleavage takes place. Because the cytoplasm of the zygote is not homogeneous, these first cell divisions result in the differential distribution of nutrients and informational molecules among the cells of the early embryo. In most animals, cleavage proceeds with rapid DNA synthesis and mitosis, but no cell growth and little gene expression. The embryo becomes a ball of smaller and smaller cells. This ball forms a central cavity called a blastocoel (a process called blastulation), at which point it is called a blastula. The individual cells are called blastomeres.

Cleavage, and therefore the formation of the blastula, is influenced in different species by two major factors. First, in some species, massive amounts of nutrients, or yolk, are stored in the egg. The yolk influences the pattern of cell divisions by impeding the formation of cleavage furrows between the daughter cells. Second, proteins and mRNAs stored in the egg by the mother guide the formation of mitotic spindles and the timing of cell divisions.

Fertilization

(c) Dorsal enrichment inhibitor

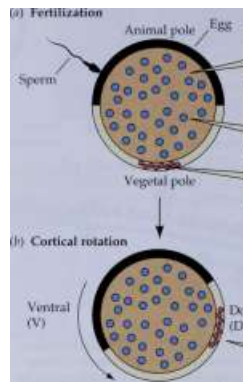
I

(d) Dorsal inhibition of GSK-3

\

(c) Dorsal enrichment of β -catenin

I



(β -catenin (orange)

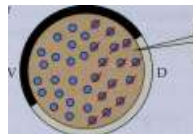
is distributed throughout
cytoplasm.

GSK-3 (blue), which degrades β -catenin, is also distributed throughout cytoplasm.

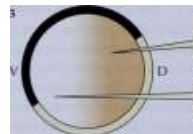
A protein that inhibits GSK-3 is contained in vegetal pole vesicles.

Dorsal (D)

Vesicles in vegetal pole move on microtubule tracks to side opposite sperm entry.



The vesicles release GSK-inhibiting protein...



...so GSK-3 cannot degrade β -catenin on the dorsal side...

J ...but does degrade it on the ventral side.



Thus there is a higher β -catenin concentration in the dorsal cells of the early embryo.

43.6 Cytoplasmic Factors Set Up Signaling Cascades

Cytoplasmic movement changes the distributions of critical developmental signals. In the frog zygote, the interaction of the protein kinase GSK-3, its inhibitor, and the protein β -catenin are crucial in specifying the dorsal-ventral (back-belly) axis of the embryo.

FERTILIZED EGG

2-CELL STAGE

4-CELL STAGE

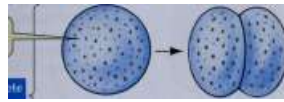
(a) Sea urchin (lateral view)

Yolk platelets are evenly distributed.

Complete cleavage

(b) Frog

(lateral view)



Yolk is concentrated at the vegetal pole.

|<—0.15mm—5>|

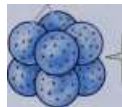


Animal pole

8-CELL STAGE

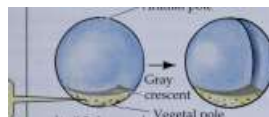
Blastomeres

Vegetal pole



Early cleavage results in blastomeres of similar size.

Animal pole



^0.5-1 mm ^ Ve 8 etal P ole



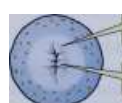
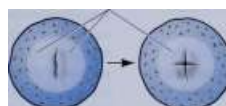
Blastomeres at the animal pole are smaller, and those at the vegetal pole are larger.

(c) Chick

(view from top)

Incomplete cleavage

Blastomeres



c - \

The embryo develops

on top of the yolk as a

disc of cells.

Cleavage is incomplete.

(< 25 mm— ^

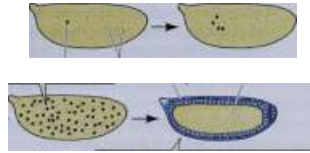
(d) *Drosophila* (lateral section)

Superficial cleavage

Multiple nuclear divisions result in a zygote with many nuclei.

Single

cell layer Yolk core



Nucleus Yolk | < 0.5 mm >|

The amount of yolk influences cleavage

When the yolk content of the egg is sparse, there is little interference with cleavage furrow formation, and all the daughter cells are of similar size; the sea urchin egg provides an example (Figure 43.7a). More yolk means more resistance to cleavage furrow formation; therefore, cell divisions progress more rapidly in the animal hemisphere than in the vegetal hemisphere, where the yolk is concentrated. As a result, the cells derived from the vegetal hemisphere are fewer and larger; the frog egg provides an example (Figure 43.7b).

In spite of this difference between sea urchin and frog eggs, the cleavage furrows completely divide the egg mass in both cases; thus these animals are said to have complete cleavage. In contrast, in an egg, such as the chicken egg, that contains a lot of yolk, the cleavage furrows do not penetrate the yolk. As a result, cleavage is incomplete, and the embryo forms as a disc of cells, called a blastodisc, on top of the yolk mass (Figure 43.7c). This type of cleavage, called incomplete cleavage, is common in fishes, reptiles, and birds.

Another type of incomplete cleavage, called superficial cleavage, occurs in insects such as the fruit fly (*Drosophila*).

The nuclei migrate to the periphery, and plasma membranes form between them.

43.7 Patterns of Cleavage in Four Model Organisms

Patterns of early embryonic development reflect differences in the way the egg cytoplasm is organized.

In the insect egg, the massive yolk is centrally located (Figure 43.7d). Early in development, cycles of mitosis occur without cytokinesis. Eventually the resulting nuclei migrate to the periphery of the egg, and after several more mitotic cycles, the plasma membrane of the egg grows inward, partitioning the nuclei off into individual cells.

The orientation of mitotic spindles influences the pattern of cleavage

The positions of the mitotic spindles during cleavage are not random; rather, they are determined by cytoplasmic factors. In turn, the orientation of the mitotic spindles determines the cleavage planes and, therefore, the arrangement of the daughter cells.

If the mitotic spindles of successive cell divisions form at right angles or parallel to the animal-vegetal axis of the zygote, the cleavage pattern is radial, as in the sea urchin and the frog (see Figure 43.7a and b). The orientations of successive mitotic spindles in mammals are also at right angles or

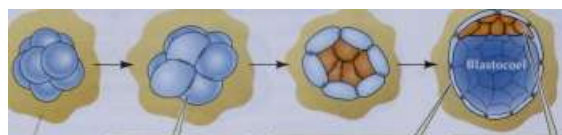
758 CHAPTER FORTY-THREE

Early 8-cell stage

Later 8-cell stage (compaction)

16 Cells

Blastocyst (32 cells)



Zona pellucida

Tight junctions form between cells.

The trophoblast will become part of the placenta.

The inner cell mass will form the embryo.

43.8 A Blastocyst Forms in Mammals

Starting at the late 8-cell stage, the mammalian embryo undergoes compaction of its cells, resulting in a blastocyst—a dense inner cell mass on top of a hollow blastocoel, surrounded by a trophoblast.

parallel to the animal-vegetal axis, but the sequence is different from that in sea urchins, resulting in a pattern called rotational cleavage. In mollusks, the successive mitotic spindles are not at right angles; the resulting pattern has a twist, and is called spiral cleavage. The coiling of snail shells is an expression of this form of cleavage.

Cleavage in mammals is unique

The early development of placental mammals is quite different from that of the other species we have discussed. The zygote of a placental mammal produces both an embryo and the elaborate extraembryonic structures that serve as the interface between the embryo and the maternal uterus. Cell divisions are slower and genes are expressed during cleavage in placental mammals. As a result, proteins encoded by the genes of the embryo play a role in cleavage. In other species, cleavage is directed almost entirely by molecules that were present in the egg.

As in other animals that have complete cleavage, the early cell divisions in a mammalian zygote produce a loosely associated ball of cells. However, at about the 8-cell stage, the behavior of the cells changes. They suddenly maximize their surface contact with each other, form tight junctions, and become a very compact mass of cells.

At the transition from the 16-cell to the 32-cell stage, the cells separate into two groups. The innermost cells form the inner cell mass that will become the embryo, while the outermost cells become an encompassing sac called the trophoblast, which will become part of the placenta. The trophoblast cells secrete fluid, thus creating a cavity with the inner cell mass at one end (Figure 43.8). At this stage, the mammalian embryo is called a blastocyst to distinguish it from the blastula of other animals.

When the blastocyst reaches the uterus, the trophoblast adheres to the uterine wall, beginning the process of implantation that embeds the embryo in the endometrium. In humans, implantation begins about the sixth day after fertilization. Implantation must not occur as the blastocyst moves down the oviduct to the uterus, or the result will be an ectopic or tubal pregnancy—a very dangerous condition. Early implantation is normally prevented by the zona pellucida, which remains around the cleaving ball of cells. At about the time the blastocyst reaches the uterus, it "hatches" from the zona pellucida, and implantation can occur.

Specific blastomeres generate specific tissues and organs

Cleavage in all species results in a repackaging of the cytoplasm of the egg into a large number of small cells surrounding a central cavity. Little cell differentiation has occurred during cleavage, and in most nonmammalian species none of the genome of the embryo has been expressed. Nevertheless, cells in different regions of the blastula possess different complements of the nutrients and informational molecules that were present in the egg.

The blastocoel prevents cells from different regions of the blastula from interacting, but that will soon change. During the next stage of development, the cells of the blastula will move around and come into new associations with one another, communicate instructions to one another, and begin to differentiate.

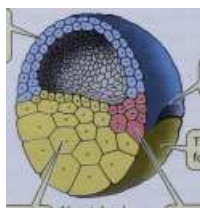
In many animals, the movements of the blastomeres are so regular and well orchestrated that it is possible to label a specific blastomere and identify the tissues and organs that form from its progeny. Such labeling experiments produce fate maps of the blastula (Figure 43.9).

Blastomeres become determined—committed to specific fates—at different times in different species. In some

Animal pole

Ectoderm will form epidermal layer of skin.

Endoderm will form the lining of the gut, the liver, and the lungs.



Dorsal lip of blastopore will form notochord.

The neural ectoderm will form the nervous system.

Vegetal pole

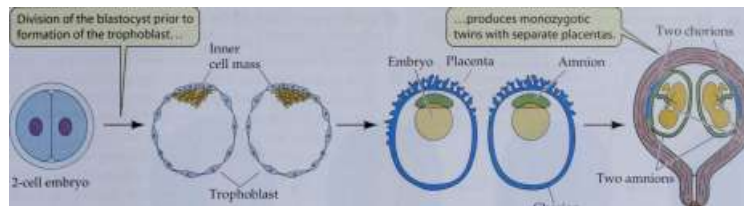
Mesoderm will form muscle, bone, kidneys, blood, gonads, and connective tissues.

43.9 Fate Map of a Frog Blastula

The colors indicate the portions of the *Xenopus* blastula that will form the three germ layers, and subsequently the frog's tissues and organs.

Division of the blastocyst prior to formation of the trophoblast...

...produces monozygotic twins with separate placentas.



Embryos

2-cell embryo

Trophoblast

43.10 Twinning in Humans

In humans, monozygotic (identical) twins result when groups of cells in the blastula become physically separated and both groups produce embryos.

species, such as roundworms and clams, blastomeres at the 8-cell stage are already determined. If one of these blastomeres is experimentally removed, a particular portion of the embryo will not form. This type of development has been called mosaic development because each blastomere seems to contribute a specific set of "tiles" to the final "mosaic" that is the adult animal. In contrast, other species, such as sea urchins, frogs, and vertebrates, have regulative development: The loss of some cells during cleavage does not affect the developing embryo because the remaining cells compensate for the loss.

If some blastomeres can change their fate to compensate for the loss of other cells during cleavage and blastula formation, are these cells capable of forming an entire embryo? To a certain extent they are. During cleavage or early blastula formation, if the blastomeres are physically separated into two groups, both groups can produce complete embryos (Figure 43.10). Since the two embryos come from the same zygote, they will be monozygotic twins —genetically identical. Non-identical twins occur when two separate eggs are fertilized by two separate sperm. Thus, while identical twins are always of the same sex, non-identical twins have a 50 percent chance of being the same sex.

Chorion

► The cells remaining on the outside become the outer germ layer, the ectoderm, and give rise to the epidermis and the nervous system.

► Other cells migrate between these two layers to become the middle germ layer, or mesoderm, which will contribute tissues to many organs, including bones, muscles, liver, heart, and blood vessels.

Some of the most challenging and interesting questions in animal development have concerned what directs the cell movements of gastrulation and what is responsible for the resulting patterns of cell differentiation and organ formation. In the past 20 years scientists have answered many of these questions at the molecular level. In the discussion that follows, we consider the similarities and differences of gastrulation in sea urchins, frogs, reptiles, birds, and mammals. We also review some of the exciting discoveries about the mechanisms underlying these phenomena.

Involution of the vegetal pole characterizes gastrulation in the sea urchin

The sea urchin blastula is a simple, hollow ball of cells that is only one cell layer thick. The end of the blastula stage is marked by a dramatic slowing of the rate of mitosis, and the beginning of gastrulation is marked by a flattening of the vegetal hemisphere (Figure 43.11). Some cells at the vegetal pole bulge into the blastocoel, break free, and migrate into the cavity. These cells become primary mes-

Gastrulation: Producing the Body Plan

The blastula is typically a fluid-filled ball of cells. How does this ball of cells become an embryo, made up of multiple tissue layers, with head and tail ends and dorsal and ventral sides? Gastrulation is the process by which layers of tissue, called germ layers, form and take specific positions relative to one another (Figure 43.9; Table 43.1). The resulting spatial relations between tissues make possible inductive interactions: exchanges of signals among tissues that trigger differentiation and organ formation.

During gastrulation, the animal body forms three germ layers:

► Some blastomeres move as a sheet to the inside of the embryo, creating an inner germ layer, the endoderm, which will

give rise to gut tissues.

43.1

Fates of Embryonic Germ Layers in Vertebrates"

GERM LAYER

FATE

Ectoderm Brain and nervous system; lens of eye; inner ear; lining of mouth and of nasal canal; epidermis of skin; hair and nails; sweat glands, oil glands, milk secretory glands

Mesoderm Skeletal system (bones, cartilage, notochord); gonads; muscle; outer coverings of internal organs; dermis of skin; circulatory system (heart, blood vessels, blood cells); kidneys

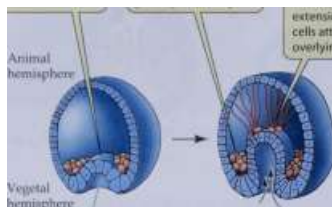
Endoderm Inner linings of gut; respiratory tract (including lungs); liver; pancreas; thyroid; urinary bladder

" The final structures are complex, containing cells from more than one germ layer. Interactions among tissues are usually important in determining the composition and structure of an organ.

760 CHAPTER FORTY-THREE

Q Some cells move inward to form the archenteron.

o Other blastoderm cell; ingress, becoming primary mesenchyme.



D More cells break free,

forming secondary

mesenchyme. Thin

extensions of these cells attach to the overlying ectoderm.

Vegetal hemisphere /

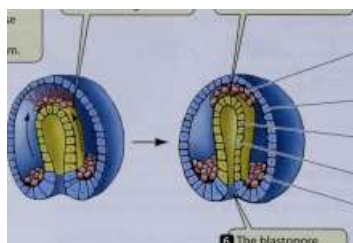
Blastopore

43.11 Gastrulation in Sea Urchins

During gastrulation, cells move to new positions and form the three germ layers from which all adult tissues develop.

Q Cells in the wall of the archenteron elongate and rearrange.

o The mouth forms where the archenteron meets the ectoderm.



The blastopore forms the anus of the mature animal.

Secondary mesenchyme

Ectoderm

Endoderm

Archenteron

Primary mesenchyme

enchyme cells—cells of the middle germ layer, the mesoderm. (The word "mesenchyme" means "a loosely organized group of cells," in contrast to cells formed into a tightly packed sheet.)

The flattening at the vegetal pole becomes an involution, as if someone were poking a finger into a hollow ball. The cells that involute become the endoderm and form the primitive gut, the archenteron. At the tip of the archenteron more cells break free, entering the blastocoel to form more mesoderm, the secondary mesenchyme.

The archenteron continues to move inward, partly because of changes in the shapes of its cells and partly because it is pulled by secondary mesenchyme cells. These cells, attached to the tip of the archenteron, send out extensions that adhere to the overlying ectoderm and contract. Where the archenteron eventually makes contact with the ectoderm, the mouth will form, and the blastopore, the opening created by the invagination of the vegetal pole, will become the anus of the animal.

What mechanisms control the various cell movements of sea urchin gastrulation? The immediate answer is that specific properties of particular cells change. For example, some vegetal cells migrate into the blastocoel to form the primary mesenchyme because they lose their attachments to neighboring cells. Once they bulge into the blastocoel, they move by extending long processes called filopodia along an extracellular matrix of proteins that is laid down by the ectodermal cells lining the blastocoel.

A deeper understanding of gastrulation requires that we discover the molecular mechanisms whereby certain cells

43.12 Vegetal Pole Cells Contain Essential Cytoplasmic Factors

If a sea urchin blastula is divided into animal pole and vegetal pole cells, only the vegetal pole cells have the capacity to develop normally. If a few vegetal pole cells are added to a mass of animal pole cells, normal development can occur.

EXPERIMENT

Question: Are cytoplasmic factors necessary for development divided up during cleavage in sea urchins?

Normal development

Animal hemisphere



Vegetal hemisphere

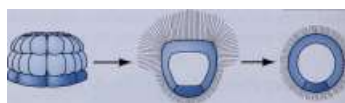
Experiment 1

METHOD Remove the vegetal half.



Normal larva

RESULT



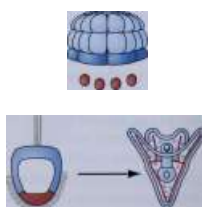
Experiment 2

METHOD Remove all vegetal cells

except the bottommost ones.

Abnormal larva

RESULT



Complete larva

Conclusion: Cytoplasmic factors essential for initiating the normal developmental cascade are localized into the vegetal pole cells during cleavage.

of the blastula develop properties different from those of others. Cleavage divides up the cytoplasm of the egg in a very systematic way. The sea urchin blastula at the 64-cell stage can be viewed as consisting of tiers of cells. As in the frog blastula, the top is the animal pole and the bottom the vegetal pole. If different tiers of blastula cells are separated, they show different developmental potentials (Figure 43.12). Only cells from the vegetal pole are capable of initiating the development of a complete larva.

It has been proposed that transcriptional regulatory proteins are unevenly distributed in the egg cytoplasm and therefore end up in particular groups of cells as cleavage progresses. Possibly, transcription factors found in the lowest vegetal cells become active in early cleavage. Indeed, one of these factors appears to be P-catenin, the transcription factor that is differentially distributed in the frog zygote. The genes activated by β -catenin seem to produce proteins that set up a signaling cascade that initiates the processes of cell determination and differentiation. Let's turn now to gastrulation in the frog, in which a number of key signaling molecules have been identified.

Animal pole

Q Gastrulation begins when cells just below the center of the gray crescent move inward to form the dorsal lip of the future blastopore.



Blastocoel

Bottle cells

Dorsal lip of blastopore

Vegetal pole

Blastocoel displaced

o Cells of the animal pole spread out, pushing surface cells below them toward and across the dorsal lip. Those cells involute into the interior of the embryo, where they form the endoderm and mesoderm.



Archenteron

f

Gastrulation in the frog begins at the gray crescent

Amphibian blastulas have considerable yolk and are more than one cell layer thick; therefore, gastrulation is more complex in the frog than in the sea urchin. Gastrulation begins when certain cells in the gray crescent region of the blastula change their shape and their cell adhesion properties. These are the cells that received high concentrations of β -catenin in their cytoplasm and therefore made certain proteins that cells in other parts of the blastula did not make. In some way, this pattern of gene expression causes the bodies of these cells to bulge into the blastocoel while they remain attached to the outer surface by slender necks. Because of their shape, these cells are called bottle cells.

As the bottle cells move into the interior of the blastula, they appear to pull other surface cells in after them (Figure 43.13). This process creates a lip over which a sheet of cells moves into the blastocoel. The first involuting cells are the prospective endoderm, and they form the primitive gut, the archenteron. These cells also bring into the blastocoel the cells that will form the mesoderm. The initial site of involution is called the dorsal lip of the blastopore, and it plays a central role in vertebrate development.

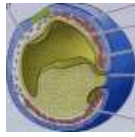
As gastrulation proceeds, cells from all over the surface of the blastula move toward the site of invagination. This movement of cells toward the blastopore is called epiboly. The dorsal lip of the amphibian blastopore widens and eventually forms a complete circle surrounding a plug of yolk-rich cells. As cells continue to move in through the blastopore, the archenteron grows and gradually displaces the blastocoel.

§J Involution creates the archenteron and destroys the blastocoel. The blastopore lip forms a circle, with cells moving to the interior all around the blastopore; the yolk plug is visible through the blastopore.



Mesenchyme

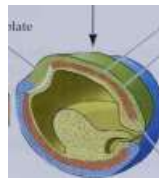
Continued development gives rise to a notochord derived from mesoderm.



Neural plate of brain

t

The beginnings of the nervous system (green) are derived from ectoderm.



Endoderm

Archenteron

Mesoderm

Dorsal lip of blastopore

Ectoderm

Notochord

Endoderm

Dorsal lip of blastopore

Yolk plug

Ventral lip of blastopore

Ectoderm Endoderm

Notochord

Dorsal lip of blastopore

Mesoderm

Mesenchyme Neural plate

rn



Notochord

43.13 Gastrulation in the Frog *Xenopus*

The colors in this diagram are matched to those in the frog fate map (Figure 43.9).

As gastrulation comes to an end, the embryo consists of three germ layers: ectoderm on the outside, endoderm on the inside, and mesoderm in the middle. The embryo also has a dorsal-ventral and anterior-posterior organization. Most importantly, however, the fates of specific regions of

EXPERIMENT

Question: Are cytoplasmic factors necessary for development localized in the fertilized egg?

Experiment 1

Experiment 2



METHOD

Using a baby's hair, the zygote is constricted along the plane of first cleavage

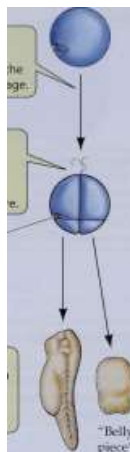
One constriction bisects the gray crescent; the other restricts it to one blastomere.

v J

Gray crescent

RESULTS

Only those blastomeres with gray crescent material develop normally.



Normal Normal

Normal

Conclusion: Cytoplasmic factors in the gray crescent are crucial for normal development.

43.14 Spemann's Experiment

Spemann's research revealed that gastrulation and subsequent normal development in salamanders depended on cytoplasmic factors localized in the gray crescent.

the i ndoderm, mesoderm, and ectoderm have become "determined" such that they will differentiate into specific tissue typt
The discovery of determination, our next topic, is one of the ost exciting stories in animal development.

The dorsal lip of the blastopore organizes formation of tt embryo

Early in the 1900s, German biologist Hans Spemann was studying the develop, lent of salamander eggs. He was interested in finding out . ether nuclei remain totipotent — capable of directing the <_, -elopment of a complete embryo—during cleavage

and blastula formation. With great patience and dexterity, he formed a loop from a single human baby hair to constrict fertilized eggs.

When Spemann's loop bisected the gray crescent, the cells on both sides of the constriction developed into complete embryos (Figure 43.14, left). But when the gray crescent was on only one side of the constriction, only that side

gastrulated and developed into a complete embryo. The side lacking gray crescent material became a clump of undifferentiated cells Spemann called the "belly piece" (Figure 43.14, right). Spemann thus hypothesized that cytoplasmic factors contained in the region of the gray crescent are necessary for gastrulation and thus for the development of a normal organism.

To test his hypotheses, Spemann and his student Hilde Mangold conducted a series of delicate tissue transplantation experiments. They transplanted pieces of early gastrulas to various locations on other gastrulas. Guided by fate maps (see Figure 43.9), they were able to take a piece of ectoderm they knew would develop into epidermis and transplant it to a region that normally becomes nervous system, and vice versa (Figure 43.15).

When they did these transplants in early gastrulas, the transplanted pieces always developed into tissues that were appropriate for the location where they were placed. Donor presumptive epidermis (that is, cells destined to become skin in their original location) developed into host nervous system, and donor presumptive neural ectoderm developed into host skin. Thus, the fates of the transplanted cells had not been determined before the transplantation.

In late gastrulas, however, the same experiment yielded opposite results. Donor presumptive epidermis produced patches of skin cells in the host nervous system, and donor presumptive neural ectoderm produced neurons in the host skin. Something had occurred during gastrulation to determine the fates of the embryonic cells. In other words, as Spemann had hypothesized, the path of differentiation a cell would follow was determined during gastrulation.

Then Spemann and Mangold did an experiment that produced momentous results: they transplanted the dorsal lip of the blastopore. When this small piece of tissue was transplanted into the presumptive belly area of another gastrula, it stimulated a second site of gastrulation, and another whole embryo formed belly-to-belly with the original embryo. The dorsal lip of the blastula was apparently capable of inducing the formation of an embryo. Thus, Spemann and Mangold called the dorsal lip of the blastopore the primary embryonic organizer.

The primary embryonic organizer has been studied intensively, and we are beginning to understand the molecular mechanisms involved in its action. The distribution of β -catenin corresponds to the location of the primary embryonic organizer, so it is a candidate molecule for initiating organizer activity. Correlation is not enough, however. To prove that a molecule is an inductive signal, it has to be shown that it is both necessary and sufficient for the proposed effect. In other words, the effect should not occur where expected if the candidate molecule is eliminated (necessity), and the candidate molecule must be capable of inducing the effect where it would otherwise not occur (sufficiency).

The criteria of necessity and sufficiency have been satisfied for β -catenin. If β -catenin transcripts are depleted by injections of antisense RNA into the egg (see Chapter 17), gastrulation does not occur. If β -catenin is experimentally

EXPERIMENT A

Question: When is presumptive epidermis determined to follow a particular developmental path?

Experiment 1

METHOD Transplant neural ectoderm in early gastrula.

Experiment 2

METHOD Transplant neural ectoderm in late gastrula.

Presumptive neural ectoderm

--* Presum P tive RESULTS / ^" ~* ^p^ epidermis

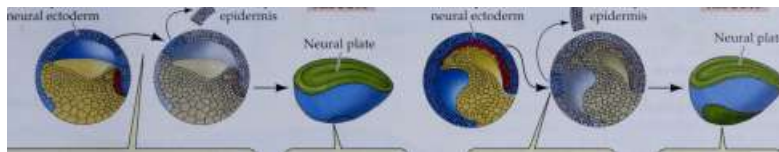
Neural plate

Presumptive neural ectoderm

Presumptive RESULTS

epidermis

Neural plate



At the early gastrula stage, transplant of neural ectoderm to presumptive epidermis...

...develops into skin at the new location.

At the late gastrula stage, a similar transplant...

...produces a second neural plate.

Conclusion: During gastrulation, presumptive neural ectoderm becomes determined to develop into nervous system.

EXPERIMENT B

Question: Can some cells induce other cells to follow a particular developmental path?

METHOD Transplant blastopore lip to opposite side of embryo.

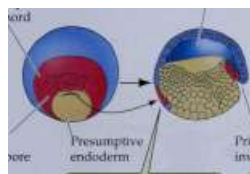
Presumptive notochord

Dorsal

blastopore

lip

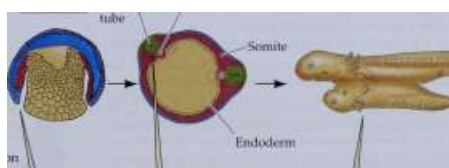
Blastocoel



RESULTS Neural Notochord tube

Presumptive endoderm

Primary invagination



Transplant of early gastrula dorsal blastopore lip...

...initiates a secondary involution,...

..secondarily induced structures,...

..and a complete secondary embryo attached to the first.

Conclusion: The dorsal lip can induce other cells to participate in embryogenesis.

43.15 Tissue Transplants Reveal the Process of Determination

Tissue transplant experiments by Spemann and Mangold revealed that cell fate becomes increasingly determined during gastrulation. Transplanting the dorsal lip of the blastopore resulted in a second initiation of gastrulation and the formation of a second embryo.

overexpressed in another region of the blastula, it can induce a second axis of embryo formation, as the dorsal lip did in the Spemann-Mangold transplantation experiments. Thus, P-catenin appears to be both necessary and sufficient for the formation of the primary embryonic organizer, but it is only one component of a complex signaling cascade that is still the subject of intense investigation.

Reptilian and avian gastrulation is an adaptation to yolky eggs

The eggs of reptiles and birds have a massive yolk content, and therefore the blastulas of these species develop as a disc of cells on top of the yolk. We will use the chicken egg

to show how gastrulation occurs in a flat disc of cells rather than in a ball of cells.

Cleavage in the chick results in a flat, circular layer of cells called the blastodisc. Between the blastodisc and the yolk mass is a fluid-filled space. Some cells from the blastodisc break free and move into this space. Other cells grow into this space from the posterior margin of the blastodisc. These cells come together to form a continuous layer called the hypoblast. The overlying cells are called the epiblast. Thus, the avian blastula is a flattened structure consisting of an upper epiblast and a lower hypoblast, which are joined at the margins of the blastodisc. The blastocoel is the fluid-filled space between the epiblast and hypoblast.

764 CHAPTER FORTY-THREE

t

The posterior epiblast thickens.



Q Cells move toward the primitive streak, down through it, and forward.

The primitive streak narrows and lengthens...

...forming the primitive groove—the chick's blastopore.

Cells passing over Hensen's node form head structures and notochord.

Embryo

Yolk

Posterior

Primitive streak



Epiblast

Blastocoel

Chick embryo viewed from above

43.76 Gastrulation in Birds

Because of the large yolk mass in bird and reptile eggs, these embryos display a pattern of gastrulation very different from that of sea urchins and amphibians.

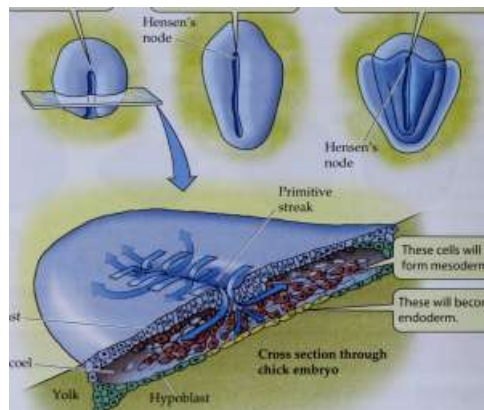
Gastrulation begins with a thickening of a posterior region of the epiblast caused by the movement of cells toward the midline and then forward along the midline (Figure 43.16). The result is a midline ridge called the primitive streak. A depression called the primitive groove forms along the length of the primitive streak. The primitive groove becomes the blastopore as cells migrate through it into the blastocoel to become endoderm and mesoderm.

In the avian embryo, no archenteron forms, but the prospective endoderm and mesoderm migrate forward to form gut and other structures. At the extreme forward end of the primitive groove is a thickening called Hensen's node, which is the equivalent of the dorsal lip of the amphibian blastopore. In fact, many signaling molecules that have been identified in the frog organizer are also expressed in Hensen's node. Cells passing over Hensen's node become determined to differentiate into the tissues and structures that make up the head and the dorsal midline of the embryo.

Mammals have no yolk, but retain the avian-reptilian gastrulation pattern

Mammals and birds both evolved from reptilian ancestors, so it is not surprising that they share patterns of early development, even though the eggs of placental mammals have no yolk. Earlier we described the development of the mammalian trophoblast and the inner cell mass, which is the equivalent of the avian epiblast. Keeping avian gastrulation in mind, think of the mammalian inner cell mass as sitting on top of an imaginary body of yolk (Figure 43.17).

As in avian development, the inner cell mass splits into an upper layer called the epiblast and a lower layer called the hypoblast with a fluid-filled cavity, or blastocoel, between them. The embryo will form from the epiblast, and the hypoblast will contribute to the extraembryonic membranes. The epiblast also contributes to the extraembryonic



These cells will
form mesoderm.

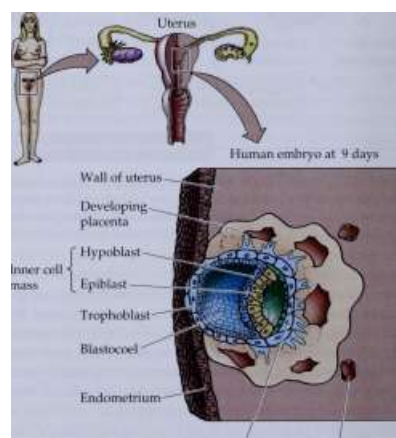
<

These will become endoderm.

Cross section through chick embryo

Hypoblast

Uterus



Inner cell -s

mass [] Epiblast

Trophoblast Blastocoel

Endometrium

Amnion

Blood vessel

43.77 A Human Blastocyst before Gastrulation

The mammalian inner cell mass becomes the epiblast; it can be compared to the avian embryo by picturing it sitting on top of a mass of imaginary yolk.

%;£

membranes; specifically, it splits off an upper layer of cells that will form the amnion. The amnion will grow to surround the developing embryo as a sac filled with amniotic fluid.

Gastrulation occurs in the mammalian epiblast just as it does in the avian epiblast. A primitive groove forms, and epiblast cells migrate through the groove to become layers of endoderm and mesoderm. The cells migrating over Hensen's node become the cells that form dorsal structures such as the brain and spinal cord.

Neurulation: Initiating the Nervous System

Gastrulation produces an embryo with three germ layers that are positioned to influence one another through inductive tissue interactions. During the next phase of development, called organogenesis, many organs and organ systems develop simultaneously and in coordination with one another. An early process of organogenesis that is directly related to gastrulation is neurulation, the initiation of the nervous system. We will examine this event in the amphibian embryo, but it occurs in a similar fashion in reptiles, birds, and mammals. Many of the genes involved are highly conserved all the way from worms to humans.

The stage is set by the dorsal lip of the blastopore

The cells that pass through the dorsal lip of the blastopore and move anteriorly in the blastocoel during gastrulation are determined to become mesoderm. The dorsal mesoderm closest to the midline is further determined to become chordomesoderm, which forms a rod along the dorsal midline. This rod, called the notochord, gives structural support to the developing embryo. The notochord eventually will be replaced by the vertebral column, but after gastrulation it induces the overlying ectoderm to begin forming the nervous system. Neurulation involves the formation of an internal tube from an external sheet of cells. The first signs of neurulation are flattening and thickening of the ectoderm overlying the notochord; this thickened area forms the neural plate (Figure 43.18). The edges of the neural plate that run in an anterior-posterior direction continue to thicken to form ridges or folds. Between the folds a groove forms and deepens as the folds roll over it to converge on the midline. The folds fuse, forming a cylinder, the neural tube, and a continuous overlying layer of epidermal

43.18 Neurulation in the Frog

Continuing the sequence from Figures 43.9 and 43.13, these drawings outline the development of the frog's neural tube. The mid-sagittal section (fc>) shows the development of the notochord and its position relative to the neural tube.

At the start of neurulation:

The neural plate, which forms from ectoderm above the notochord, is well defined.

(a) Dorsal

surface view

Blastopore

In the middle of neurulation:

As the edges of the neural plate move upward and grow toward one another, the center of the plate sinks, forming the neural groove.

Late in neurulation:

When the edges of the neural plate grow together and fuse, a hollow cylinder forms and detaches from the ectoderm to become the neural tube.



Neural fold

Neural plate Blastopore



Neural groove



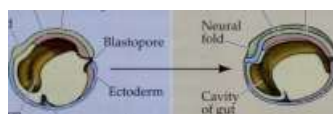
Fused neural folds

(b) Midsagittal section

Notochord Neural plate Neural fold \

Notochord

Archenteron



Neural plate

Blastopore

Neural tube

Remnant of the blastocoel



(c) Transverse section

Notochord Neural plate

Neural fold

Endoderm



Archenteron

Mesoderm Ectoderm

Neural groove Notochord /

Cavity Jf* cfV Neural plate of gut .



Notochord

Cavity of gut

Neural tube

Mesoderm



ectoderm. The neural tube develops bulges at the anterior end, which become the major divisions of the brain; the rest of the tube becomes the spinal cord.

Failure of the neural tube to develop normally can result in serious birth defects. If the neural tube fails to close in a posterior region, the result is a condition known as spina bifida. If it fails to close at the anterior end, an infant can develop without a forebrain—a condition called anencephaly. Whereas several genetic factors have been identified that can cause neural tube defects, there are also environmental factors, including dietary ones. The incidence of neural tube defects used to be about 1 in 300 live births, but we now know that this incidence can be cut in half if pregnant women have an adequate amount of folic acid (a B vitamin) in their diets.

Body segmentation develops during neurulation

Like the fruit flies whose development we traced in Chapter 16, vertebrates have a body plan consisting of repeating segments that are modified during development. These segments are most evident as the repeating patterns of vertebrae, ribs, nerves, and muscles along the anterior-posterior axis.

As the neural tube forms, mesodermal tissues gather along the sides of the notochord to form separate blocks of cells called somites (Figure 43.19). The somites produce cells that will become vertebrae, ribs, and muscles of the trunk and limbs.

The nerves that connect the brain and spinal cord with tissues and organs throughout the body are also arranged segmentally. The somites help guide the organization of these peripheral nerves, but the nerves are not of mesodermal origin. When the neural tube closes, cells adjacent to the line of closure break loose and migrate inward between the epidermis and the somites and under the somites. These cells, called neural crest cells, give rise to a number of structures, including the peripheral nerves, which grow out to the body tissues and back into the spinal cord.

As development progresses, the segments of the body become different. Regions of the spinal cord differ, regions of the vertebral column differ in that some vertebrae grow ribs of various sizes and others do not, forelegs arise in the anterior part of the embryo, and hind legs arise in the posterior region. How does a somite in the anterior part of a mouse embryo "know" to produce forelegs rather than hind legs?

Central to the process of anterior-posterior determination and differentiation are homeotic genes (see Chapter 16). We have seen how these genes control body segmentation in *Drosophila*. In the mouse, four families of similar genes, called homeobox or Hox genes, control differentiation along the anterior-posterior body axis.

Each family of mammalian Hox genes resides on a different chromosome and consists of about 10 genes. What is remarkable is that the temporal and spatial expression of these genes follows the same pattern as their linear order on their chromosomes. As a result, different segments of

2-day chick embryo

Neural tube

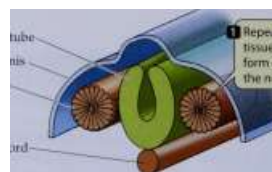
Epidermis

Somite

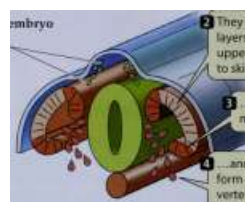
Notochord

4-day chick embryo

Neural crest cells



Repeating blocks of tissue called somites form on either side of the neural tube.

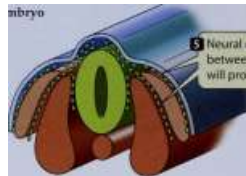


They divide into three layers of cells. The upper will contribute to skin,...

...the middle to muscles,...

and the lower will form cartilage of the vertebrae and ribs.

7-day chick embryo



Neural crest cells migrate between these layers and will produce nerves.

43.19 The Development of Body Segmentation

Repeating blocks of tissue called somites form on either side of the neural tube. Skin, muscle, and bone form from the somites.

the embryo receive different combinations of Hox gene products, which serve as transcription factors (Figure 43.20). What causes the linear, sequential expression of Hox genes is unclear.

Whereas Hox genes give cells information about their positional location on the anterior-posterior body axis, other genes give information about dorsal-ventral position. Tissues in each segment of the body differentiate according to their dorsal-ventral location. In the spinal cord, for example, sensory connections develop in the dorsal region and motor connections develop in the ventral region. In the somites, dorsal cells develop into skin and muscle and ventral cells develop into cartilage and bone.

An example of a gene that provides dorsal-ventral information in vertebrates is sonic hedgehog, which is expressed in the mammalian notochord and induces cells in the overlying neural tube to follow fates characteristic of ventral spinal cord cells. (As with the Hox genes, sonic hedgehog is homologous to a *Drosophila* gene, which is known simply as hedgehog.)

A family of homeobox genes, the Pax genes, play many roles in nervous system and somite development. One of these genes, Pax3, is expressed in neural tube cells that develop into dorsal spinal cord structures. Sonic hedgehog represses the expression of Pax3, and their interaction is one

(a)

t

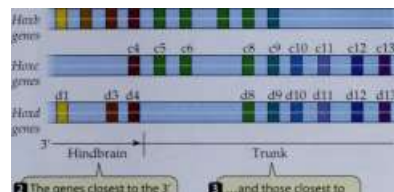
Hox genes are clustered on four chromosomes.

Hoxa genes

a1 a2 a3 a4 a5 a6 a7 a9 a10 all

b1 b2 b3 b4 b5 b6 b7 b8 b9

a13



| The genes closest to the 3' end are expressed in the anteriormost positions...

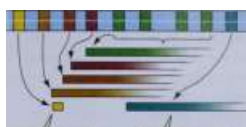
.and those closest to the 5' end are expressed more posteriorly.

(b)

Hoxb

ANIMAL DEVELOPMENT 767

b1 b2 b3 b4 b5 b6 b7 b8 b9 TT



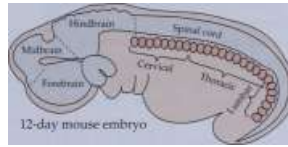
O For example, Hoxb 1 is expressed in the hindbrain...

t...an thes

.and Hoxb9 in spinal cord.

12-day mouse embryo

Expression patterns from anterior to posterior ends of embryo



43.20 Hox Genes Control Body Segmentation

The expression of Hox genes is patterned along the anterior-posterior axis of the embryo and from the 3' to the 5' ends of the chromosomes.

source of dorsal-ventral information for differentiation of the spinal cord.

With the development of body segmentation, the formation of organs and organ systems progresses rapidly. The development of an organ involves extensive inductive tissue interactions, which are a current focus of study for developmental biologists. In Chapter 16, you encountered the organogenesis of the vertebrate limb. In the next chapter you will learn about the development of the brain—another example of organogenesis.

Extraembryonic Membranes

The embryos of reptiles, birds, and mammals are surrounded by several extraembryonic membranes, which originate from the embryo but are not part of it. The ex-

5-Day embryo

traembryonic membranes play important roles in development, especially in mammals, in which they constitute the placenta that nourishes the embryo.

Four extraembryonic membranes form with contributions from all germ layers

We will use the chick to demonstrate how the extraembryonic membranes form from the germ layers created during gastrulation. The yolk sac is the first extraembryonic membrane to form, and it does so through extensions of the endodermal tissue of the hypoblast layer, which enclose the entire body of yolk in the egg (Figure 43.21, left). This yolk sac constricts at the top to create a tube that is continuous

43.21 The Extraembryonic Membranes

In birds, reptiles, and mammals, the embryo constructs four extraembryonic membranes. The yolk sac encloses the yolk, and the amnion and chorion enclose the embryo. Fluids secreted by the amnion fill the amniotic cavity, providing an aqueous environment for the embryo. The chorion, along with the allantois, mediates gas exchange between the embryo and its environment. The allantois stores the embryo's waste products.

9-Day embryo



Embryo Amnion

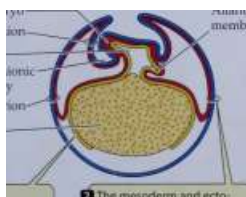
Gut

Amnionic cavity

Chorion Yolk

Allantoic membrane

| The first extraembryonic membrane is the yolk sac, which is forming in the 5-day embryo.



| The mesoderm and ectoderm extend beyond the embryo to form the chorion.

Embryo

Gut

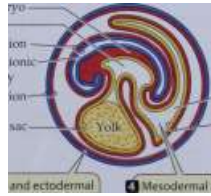
Amnion

Amnionic

cavity

Chorion Yolk sac

| The mesodermal and ectodermal layers fuse below the yolk so that the chorion lines the shell.



Allantois

Allantoic membrane

I Mesodermal and endodermal tissues form the allantois, a sac for metabolic wastes.

768 CHAPTER FORTY-THREE

Uterus

with the gut of the embryo. However, yolk does Fetus not pass through this tube. Yolk is digested by the endodermal cells of the yolk sac, and the nutrients are then transported to the embryo through blood \ essels lining the outer surface of the yolk sac.

Just as the endoderm grows out from the embryo to form the yolk sac, the ectoderm and mesoderm also extend beyond the limits of the embryo. These two layers of cells extend all along the inside of the eggshell, both over the embryo and below the yolk sac. Where they meet, they fuse, forming two membranes, the amnion and the chorion. The amnion, which is the inner membrane, surrounds the embryo, forming the amniotic cavity. The amnion secretes fluid into the cavity, providing an aqueous environment for the embryo. The outer membrane, the chorion, forms a continuous membrane just under the eggshell (Figure 43.21, right). It limits water loss from the egg and also mediates the exchange of respiratory gases between the embryo and the outside world.

The fourth membrane to form, the allantoic membrane, is another outgrowth of the embryonic endoderm. It forms the allantois, a sac for storage of metabolic wastes. The al-lantois, in combination with the chorion, is also involved in respiratory gas exchange.

Extraembryonic membranes in mammals form the placenta

In mammals, the first extraembryonic membrane to form is the trophoblast, which is already apparent by the fifth cell division (see Figure 43.8). When the blastocyst reaches the uterus and "hatches" out of its encapsulating zona pellucida, the trophoblast cells interact directly with the endometrium. Adhesion molecules expressed on the surfaces of these cells attach them to the uterine wall, and by excreting proteolytic enzymes, the trophoblast burrows into the endometrium, beginning the process of implantation (see Figure 43.17). Eventually, the entire trophoblast is within the wall of the uterus. The trophoblastic cells then send out numerous projections, or villi, to increase the surface area of contact with maternal blood.

Meanwhile, the hypoblast cells extend to form what in the bird would be the yolk sac. But there is no yolk in placental mammalian eggs, so the yolk sac contributes mesodermal tissues that interact with trophoblast tissues to form the chorion. The chorion, along with tissues of the uterine wall, produces the placenta, the organ of nutrition for the embryo (Figure 43.22).

At the same time the yolk sac is forming from the hypoblast, the epiblast produces the amnion, which grows to enclose the entire embryo in a fluid-filled amniotic cavity. The rupturing of the amnion and the loss of the amniotic fluid ("water breaking") herald the onset of labor in humans.

n allantois also develops in mammals, but its impor-

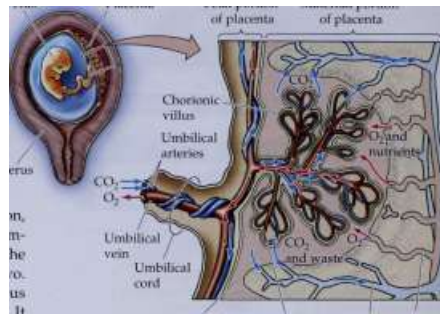
pends on how well nitrogenous wastes can be

tra; > d across the placenta. In humans the allantois is

Placenta

Fetal portion of placenta

Maternal portion of placenta



Umbilical

vein

Umb cord

Maternal blood pools in intervillous space

Fetal capillaries

Maternal venule

Maternal arteriole

43.22 The Mammalian Placenta

In most mammals, nutrients and wastes are exchanged between maternal and fetal blood in the placenta, which forms from the chorion and tissues of the uterine wall. The embryo is attached to the placenta by the umbilical cord, and embryonic blood vessels invade the placental tissue to form fingerlike villi. Maternal blood flows into the spaces surrounding the villi.

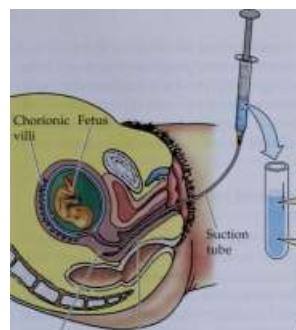
minor; in pigs it is important. In humans and other placental mammals, allantoic tissues contribute to the formation of the umbilical cord by which the embryo is attached to the chorionic placenta. It is through the blood vessels of the umbilical cord that nutrients and oxygen from the mother reach the developing fetus and wastes, including carbon dioxide and urea, are removed (see Figure 43.22).

The extraembryonic membranes provide means of detecting genetic diseases

Cells slough off of the embryo and float in the amniotic fluid that bathes it. Later in development, a small sample of the amniotic fluid may be withdrawn with a needle as the first step of a process called amniocentesis. Some of these cells can be cultured and used for biochemical and genetic analyses that can reveal the sex of the fetus, as well as genetic markers for diseases such as cystic fibrosis, Tay-Sachs disease, and Down syndrome.

If amniocentesis is performed, it is usually not until after the fourteenth week of pregnancy, and the tests require two weeks to complete. If abnormalities in the fetus are detected, termination of the pregnancy at that stage would put the mother's health at greater risk than would an earlier abortion. Therefore a newer technique, called chorionic villus sampling, is now in common use. In this test a small sample of the tissue from the surface of the chorion is taken (Figure 43.23). This test can be done as early as the eighth week of pregnancy, and the results are available in several days.

ANIMAL DEVELOPMENT 769



The sample can be used for biochemical analysis...

...or chromosomal analysis to determine sex of the fetus and presence of any chromosomal abnormalities.

Cervical canal Vagina

43.23 Chorionic Villus Sampling

Information about genetic defects can be obtained from chorionic tissues.

Human Pregnancy and Birth

Human pregnancy can be divided into three trimesters

Gestation, or pregnancy, in humans has a duration of about 266 days, or 9 months. In smaller mammals gestation is shorter—for example, 21 days in mice—and in larger mammals it is longer—for example, 330 days in horses and 600 days in elephants. To follow the temporal sequence of

events in human pregnancy, we can divide it into three trimesters of about 3 months each.

the first trimester. Implantation of the human blastocyst begins on about the sixth day after fertilization. After implantation, the placenta develops and the tissues and organs of the embryo begin to differentiate. The human heart begins to beat in week 4, and limbs form by week 8 (Figure 43.24a). Most organs have started to form by the end of the first trimester. By the end of the first trimester, the embryo looks like a miniature version of the adult, and is called a fetus.

Because the first trimester is a time of rapid cell division and differentiation, it is the period during which the embryo is most sensitive to radiation, drugs, and chemicals that can cause birth defects. An embryo can be damaged before the mother even knows she is pregnant.

Hormonal changes cause major and noticeable responses in the mother during the first trimester, even though the fetus at the end of that time is still so small that it would fit into a teaspoon. Soon after the blastocyst implants itself, it begins to secrete human chorionic gonadotropin (hCG), the hormone that stimulates the corpus luteum to continue producing estrogen and progesterone. The high levels of these hormones have negative feedback effects on the production and secretion of hypothalamic

43.24 Stages of Human Development

(a) The first trimester of pregnancy is a period of rapid cell division and differentiation. The organs and body structures of this 6-week old embryo are forming rapidly. (b) At 4 months, the fetus moves freely within its protective amniotic cavity. The fingers and toes are fully formed.



770 CHAPTER FORTY-THREE

The release of oxytocin by the pituitary gland increases the contractions of the uterus during labor and birth (a positive feedback loop)



©

Increased estrogen/ progesterone ratio

Increased contractility of uterine muscle

Increased uterine contractions

Increased pressure of fetal head on cervix

Nervous system

communicates

uterine stretching

to hypothalamus

Increased oxytocin secretion

Growth of fetus

Increased stretch of uterus

43.25 Control of Uterine Contractions and Parturition

Both mechanical and hormonal signals are involved in the onset of parturition.

and pituitary hormones (see Chapter 42). These dramatic hormonal shifts cause the well-known symptoms of pregnancy: morning sickness, mood swings, changes in the senses of taste and smell, and swelling of the breasts.

tion, shortness of breath, and swelling of the legs and ankles. Since the fourth week of pregnancy the heart of the fetus has been beating, and as the third trimester approaches its end, other internal organs mature. The digestive system begins to function, the liver stores glycogen, the kidneys produce urine, and the brain undergoes cycles of sleep and waking.

Parturition is triggered by hormonal and mechanical stimuli

Throughout pregnancy, the uterus periodically undergoes slow, weak, rhythmic contractions called Braxton Hicks contractions. These contractions become gradually stronger during the third trimester and are sometimes called false labor contractions. True labor contractions usually mark the beginning of childbirth, or parturition.

Many factors contribute to the onset of labor. Both hormonal and mechanical stimuli increase the contractility of the uterus. Progesterone inhibits and estrogen stimulates contractions of uterine muscle. Toward the end of the third trimester, the estrogen-progesterone ratio shifts in favor of estrogen. Oxytocin stimulates uterine contraction; its secretion by the pituitaries of both mother and fetus increases at the time of labor.

Mechanical stimuli come from the stretching of the uterus by the fully grown fetus and the pressure of the fetal head on the cervix. These mechanical stimuli increase the pituitary release of oxytocin, which in turn increases the activity of the uterine muscle, which causes even more pressure on the cervix. This positive feedback loop converts the weak, slow, rhythmic contractions of the uterus into stronger labor contractions (Figure 43.25).

the second trimester. During the second trimester the fetus grows rapidly to a weight of about 600 g, and the mother's abdomen enlarges considerably. The limbs of the fetus elongate, and the fingers, toes, and facial features become well formed. Fetal movements are first felt by the mother early in the second trimester, and they become progressively stronger and more coordinated. By the end of the second trimester, the fetus may suck its thumb (Figure 43.24fr).

The production of estrogen and progesterone by the placenta increases during the second trimester. As placental production of these hormones increases, the level of hCG decreases and the corpus luteum degenerates. Ovulation and menstruation are still inhibited by the steroids secreted by the placenta. Along with these hormonal changes, the unpleasant symptoms of early pregnancy usually disappear.

THE third trimester. The fetus and the mother continue to grow rapidly during the third trimester. As the fetus approaches its full size, pressure on the mother's internal organs can cause indigestion, constipation, frequent urina-



43.26 Delivery

A new person enters the world head first.

ANIMAL DEVELOPMENT 771

In the early stage of labor, the contractions of the uterus are 15 to 20 minutes apart, and each lasts 45 to 60 seconds. During this time, the contractions pull the cervix open until it is large enough to allow the baby to pass through. This stage of labor lasts an average of 12 to 15 hours in a first pregnancy and 8 hours or less in subsequent ones. Gradually the contractions become more frequent and more intense.

The second stage of labor, called delivery, begins when the cervix is fully dilated. The baby's head moves into the vagina and becomes visible from the outside (Figure 43.26). The usual head-down position of the baby at the time of delivery comes about when the fetus shifts its orientation during the seventh month. If the fetus fails to move into the head-down position, a different part of the fetus enters the vagina first, and the birth is more difficult.

Passage of the fetus through the vagina is assisted by the woman's bearing down with her abdominal and other muscles to help push the baby along. Once the head and shoulders of the baby clear the cervix, the rest of its body eases out rapidly, but it is still connected to the placenta in the mother by the umbilical cord. Delivery may take as little as a minute, or up to half an hour or more in a first pregnancy.

As soon as the baby clears the birth canal, it can start breathing and become independent of its mother's circulation. The umbilical cord may then be clamped and cut. The segment still attached to the baby dries up and sloughs off in a few days, leaving behind its distinctive signature, the belly button—more properly called the umbilicus. The detachment and expulsion of the placenta and fetal membranes takes from a few minutes to an hour, and may be accompanied by uterine contractions.

In humans, the completion of delivery is the start of many years of nurturing and care for the young organism. Many processes of development continue throughout childhood, and indeed, throughout life.

Chapter Summary

Fertilization: Interactions of Sperm and Egg

- Fertilization involves interactions between sperm and egg, including sperm activation, species-specific binding of sperm to the outer covering of the egg, the acrosomal reaction, digestion of a path through the outer covering of the egg, and fusion of sperm and egg plasma membranes. Review Figure 43.1
- The entry of the sperm into the egg triggers fast and slow blocks to polyspermy, which prevent additional sperm from entering the egg and, in mammals, signals the egg to complete meiosis and begin development. Review Figures 43.2, 43.3, 43.4
- Sperm and egg contribute differentially to the zygote. The sperm contributes a haploid nucleus and, in some species, a centriole. The egg contributes a haploid nucleus, nutrients, ribosomes, mitochondria, and informational molecules that will control the early stages of development.
- The cytoplasmic contents of the egg are not distributed homogeneously, and they are rearranged after fertilization to set up the major axes of the future embryo. Review Figures 43.5, 43.6

Cleavage: Repackaging the Cytoplasm

- In most animals, cleavage is a period of rapid cell division without cell growth or gene expression. During cleavage, the cytoplasm of the zygote is repackaged into smaller and smaller cells.
- The pattern of cleavage is influenced by the amount of yolk that impedes cleavage furrow formation and by the orientation of the mitotic spindles. The result of cleavage is a ball or mass of cells called a blastula. Review Figure 43.7
- Cleavage in mammals is unique in that cell divisions are much slower and genes are expressed early in the process. Cleavage results in an inner cell mass that becomes the embryo and an outer cell mass that becomes the trophoblast. The mammalian embryo at this stage is called a blastocyst. Review Figure 43.8
- Fate maps, which identify what tissues and organs will form from particular blastomeres, can be created for the blastula. Review Figure 43.9
- Some species undergo mosaic development, in which the fate of each cell is determined by the 8-cell stage. Other species, including vertebrates, undergo regulative development, in which cells are not determined so early and can change developmental fates. In these species, blastomeres separated at early stages can develop into complete embryos, which are then monozygotic, or identical, twins. Review Figure 43.10

Gastrulation: Producing the Body Plan

- Gastrulation involves massive cell movements that produce three primary germ layers and place cells from various regions of the blastula into new associations with one another. Review Table 43.1
- The initial step of sea urchin and amphibian gastrulation is inward movement of certain blastomeres. The site of inward movement becomes the blastopore. Cells that move into the blastula become the endoderm and mesoderm; cells remaining on the outside become the ectoderm. Cytoplasmic factors in the vegetal pole cells are essential to initiate development. Review Figure 43.11, 43.12
- Gastrulation in frogs is initiated when cells in the gray crescent move into the blastocoel. This inward migration creates the blastopore. The dorsal lip of the blastopore is a critical site for the determination of tissues. It has been called the primary embryonic organizer. Review Figures 43.13, 43.14, 43.15
- The anterior-posterior axis of the frog blastula appears to be determined by the distribution of the protein β -catenin, which activates a signaling cascade that induces the primary embryonic organizer.
- Gastrulation in reptiles and birds is different from that in sea urchins and frogs because the large amount of yolk in their eggs causes the blastula to form a flattened disc of cells. Review Figure 43.16
- Mammals have a pattern of gastrulation similar to that of birds, even though they have no yolk. Review Figure 43.17

Neurulation: Initiating the Nervous System

► Neurulation follows gastrulation. Cells that migrate over the dorsal lip of the blastopore are determined to be chordomesoderm, which forms the notochord. The notochord induces the overlying ectoderm to thicken, form parallel ridges, and fold in on itself to form a neural tube below the

772 CHAPTER FORTY-THREE

epidermal ectoderm. The nervous system develops from this neural tube. Review Figure 43.18

► The notochord and neural crest cells participate in the segmental organization of tissues called somites along the body axis. Rudimentary organs and organ systems form during this stage. Review Figure 43.19

► Four families of Hox genes determine the pattern of anterior-posterior differentiation along the body axis in mammals. Other genes, such as sonic hedgehog, contribute to dorsal-ventral differentiation. Review Figure 43.20

Extraembryonic Membranes

► The embryos of reptiles, birds, and mammals are protected and nurtured by four extraembryonic membranes. In birds and reptiles the yolk sac surrounds the yolk and provides nutrients to the embryo, the chorion lines the eggshell and participates in gas exchange, the amnion surrounds the embryo and encloses it in an aqueous environment, and the allantois stores metabolic wastes. Review Figure 43.21

► In mammals, the chorion and the trophoblast cells interact with the maternal uterus to form a placenta, which provides for nutrient and gas exchange. The amnion encloses the embryo in an aqueous environment. Review Figure 43.22

► Samples of amniotic fluid or pieces of chorion can be taken during pregnancy and analyzed for evidence of genetic disease. Review Figure 43.23

Human Pregnancy and Birth

► Pregnancy in humans can be divided into three trimesters. Early embryogenesis occurs in the first trimester; during this time, the embryo is vulnerable to damage that could lead to birth defects. Hormonal changes, including high hCG, estrogen, and progesterone levels, block further ovulation and menstruation and also cause symptoms of pregnancy.

► During the second and third trimesters the embryo grows, the limbs elongate, and organ systems mature.

► The onset of labor is due to many factors, both hormonal and mechanical, that increase contractility of uterine muscles. Oxytocin plays a major role in a positive feedback loop. Review Figure 43.25

► Birth is not the end of development, which continues throughout childhood and throughout life.

For Discussion

1. Knowing what you do about sperm-egg interactions at the time of fertilization, propose a line of research that could produce a new contraceptive method. What is the rationale for your method, and what problems will you have to overcome to make it successful?
2. If you found a protein that was localized to a small group of cells in the frog blastula, how would you determine if that protein played a role in development? Address the issues of sufficiency and necessity.
3. Find out what a chimeric mouse is and explain how it is produced.
4. During gastrulation in the chick, a gene called sonic hedgehog is expressed only on the left side of Hensen's node. What could be the significance of this expression pattern?
5. Much of the early work of describing animal development was done on sea urchins, amphibians, and chicks. Most recent work on the molecular mechanisms of animal development have been done on roundworms, fruit flies, zebrafish, and mice. Why do you think there has been a shift in the animal models used by developmental biologists?



Neurons and Nervous Systems



"On your mark." "Get set." Bang! 10.75 seconds later Marion Jones crossed the finish line 100 meters away, winning an Olympic gold medal. An amazing number of events took place in her nervous system during those 10.75 seconds. Cells in her ear converted the gunshot sound waves into bursts of electrical activity called nerve impulses, which traveled via nerve cells to a part of her brain that processes sound information. Cells from that part of the brain carried nerve impulses to another

part of the brain that commands and coordinates the movements of the body. Command cells controlling the muscles in her legs were triggered to fire bursts of nerve impulses. These cells carried the bursts of nerve impulses down the spinal cord to about the middle of her back region. There the connections were made to other command cells, sending nerve impulses to the muscles of Marion's legs and feet. Those muscles contracted and propelled her off the blocks. Each time a cell in this chain passed information to another cell, it released small packets of chemicals that stimulated the next cell to generate bursts of nerve impulses.

All of these events took about a tenth of a second. For the next 10.65 seconds, hundreds of thousands of nerve cells fired repeated bursts of nerve impulses in just the right patterns to cause the many muscles of Marion's legs, feet, arms, and torso to repeatedly contract and relax, powering the 80 or so strides it took her to reach the finish line. Each of the millions of cell-cell interactions that occurred involved a small release of chemicals from one cell and a receptor-mediated response from another cell. What amazing complexity and precision—yet running 100 meters is a simple task for the nervous system (although very few people will ever do it as fast as Marion Jones did).

The nervous system is the most complex system of the human body. As in other organs and organ systems, its basic building blocks are cells. In the nervous system, cells are arranged in networks and circuits that process information. Within those circuits resides not

On the Fast Track

Marion Jones can run fast because her nervous system processes sensory information and commands her muscles so quickly.

only the capacity to generate immediate responses to stimuli on the many sensory systems, but also the capacity to remember those stimuli and responses, to relate stimuli and responses to other experiences, and to learn from the information that is processed. Within the cells and connections of the nervous system reside our individual personalities, our egos, and our ability to love and to hate.

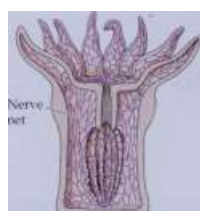
Understanding the human nervous system is probably the biggest challenge in all of biology. We start with the basic building blocks: nerve cells. This chapter describes the cells of nervous systems. It then details how nerve cells generate electric signals and conduct those signals from place to place. Finally, it explains how those signals are communicated from cell to cell and how receiving cells respond.

Nervous Systems: Cells and Functions

In multicellular animals, nerve cells, called neurons, are specialized to receive information, encircle it, and transmit it to other cells. Together with their specialized supportive cells, neurons make up nervous systems, whose functions can be described in terms of networks of interacting neurons.

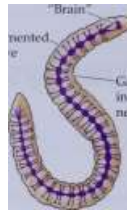
Animals receive various kinds of information from both inside and outside their bodies. This information is re-

ceived and converted, or transduced, by sensory cells (also called receptor cells) into electric signals that can be transmitted and processed by neurons. To cause behavioral or phys-



Segmented

nerve



Ganglion in ventral nerve cord

Sea anemone

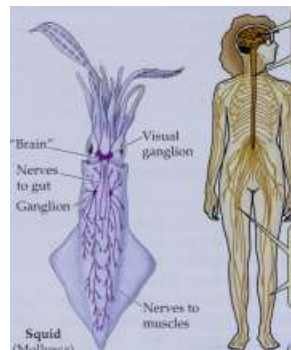
(Cnidaria)

Earthworm

(Annelida)

A nerve net serves simple behaviors such as contraction and relaxation.

In the earthworm, segmented ganglia coordinate movement and an anterior "brain" controls more complex behavior.



The human brain and spinal cord are the central nervous system...

Squid

(Mollusca) ▼

...that communicates to the cells and organs of the body via the peripheral nervous system.

Human

(Chordata)

In squid, more complex behaviors are served by collections of neurons in specialized ganglia..

44. 7 Nervous Systems Vary in Size and Complexity

As we compare animals that have increasingly complex sensory and behavioral abilities, we find information processing increasingly centralized in ganglia (collections of nerve cells) or in a brain.

biological responses, a nervous system communicates these signals to effectors, such as muscles and glands.

Nervous systems process information

A simple animal that remains fixed to its substrate, such as a sea anemone (a cnidarian), can process information with a simple network of neurons that does little more than provide direct lines of communication from sensory cells to effectors (Figure 44.1). The cnidarian's nerve net merely detects food or danger and causes its tentacles and body to extend or retract

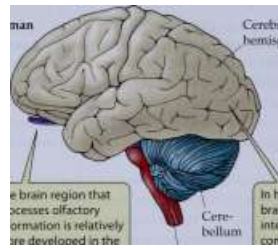
More complex animals that move around the environment and hunt for food and mates need to process and integrate larger amounts of information. Even animals such as flatworms fit this description, and their increased need for information processing is met by clusters of neurons called ganglia. Ganglia serving different functions may be distributed around the body, as in the earthworm or the squid. Frequently one pair of ganglia is larger and more recentral than the others.

In vertebrates, most of the cells of the nervous system are found in the brain and the spinal cord, the site of most information processing, storage, and retrieval (see Figure 44.1). Therefore, the brain and spinal cord are called the central nervous system (CNS). Information is transmitted from sensory cells to the CNS and from the CNS to effectors via neurons that extend or reside outside of the brain and the spinal cord; these neurons and their supporting cells are called the peripheral nervous system.

Vertebrates have highly developed central nervous systems, but they vary greatly in behavioral complexity and physiological capabilities. Figure 44.2 shows the brains of

Human

Cerebral hemispheres



f \

The brain region that

processes olfactory

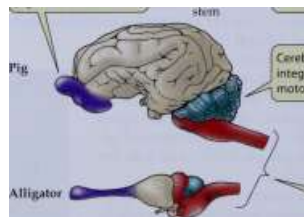
information is relatively

more developed in the

pig than in the human.

Cerebellum

Brain stem



In human, much of the brain is given over to integration of complex behaviors, learning, and memory.

Cerebellum (blue) integrates sensory and motor information.

Alligator

Shark

A shark is an "eating machine" with little complex behavior. Its brain deals primarily with sensory and motor information.

44.2 Brains Vary in Size and Complexity

The brains of four vertebrate species—all of which may have a similar body mass—show immense differences.

(a) Generalized neuron anatomy

(b) Specialized neurons

Dendrites receive information from other neurons.

Neurons with bushy dendrites collect information from many other cells.

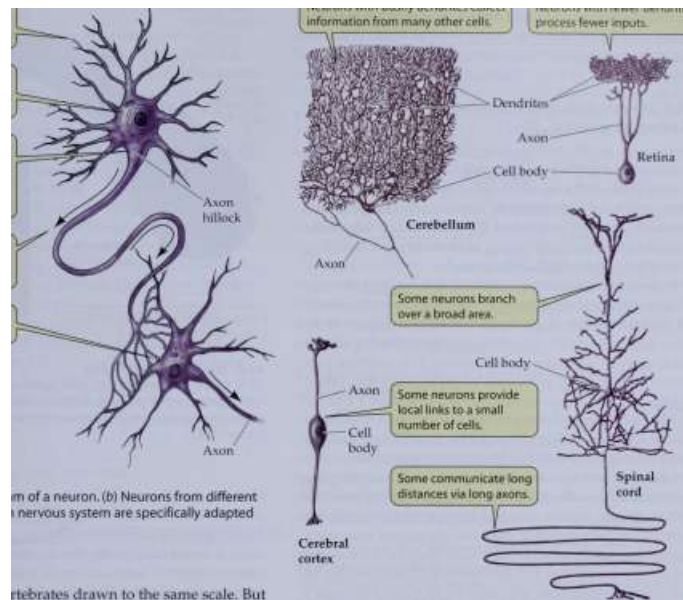
Neurons with fewer dendrites process fewer inputs.

The cell body contains the nucleus and most cell organelles.

The cell body integrates information collected by dendrites and initiates nerve impulses at the beginning of the axon.

The axon conducts nerve impulses away from the cell body.

Axon terminals synapse with a target cell.



44.3 Neurons

(a) A generalized diagram of a neuron, (b) Neurons from different parts of the mammalian nervous system are specifically adapted to their functions.

four similar-sized vertebrates drawn to the same scale. But even small nervous systems are remarkably complex. Consider the nervous systems of small spiders that have programmed within them the thousands of precise movements necessary to construct a beautiful web without prior experience.

Neurons are the functional units of nervous systems

Although nervous systems vary enormously in structure and function, neurons function similarly in animals as different as squids and humans. Their plasma membranes generate electric signals called nerve impulses or action potentials—conduct these signals from one location on a cell to the most distant reaches of that cell—a distance that can be more than a meter.

Most neurons have four regions—a cell body, dendrites, an axon, and axon terminals (Figure 44.3a)—but the variation among different types of neurons is considerable (Figure 44.3b). The cell body contains the nucleus and most of the cell's organelles. Many projections may sprout from the cell body. Most of these projections are bushlike dendrites (from the Greek dendron, "tree"), which bring information from other neurons or sensory cells to the cell body. In most neurons, one projection is much longer than the other—and

Cerebral cortex

is called the axon. Axons usually carry information away from the cell body. The length of the axon differs in different types of neurons—some axons are remarkably long, such as those that run from the spinal cord to the toes. The degree of branching of the dendrites can also be quite different among types of neurons.

Axon are the "telephone lines" of the nervous system. Information received by the dendrites can cause the cell body to generate a nerve impulse, which is then conducted along the axon to the cell that is its target. At the target cell—which can be another neuron, a muscle cell, or a gland cell—the axon divides into a spray of fine nerve endings. At the tip of each of these tiny nerve endings is a swelling called an axon terminal that comes very close to the target cell.

Where an axon terminal comes close to another cell, the membranes of both cells are modified to form a synapse. In most cases, a space or cleft only about 25 nm wide separates the two membranes at the synapse. A nerve impulse arriving at an axon terminal causes molecules called neurotransmitters stored in the axon terminal to be released. The released neurotransmitters diffuse across the synaptic cleft and bind to receptors on the plasma membrane of the target cell.

776 CHAPTER FORTY-FOUR

Thousands of synapses impinge on most individual neurons. A neuron generally receives information (synaptic inputs) from many sources before a single nerve impulse travels down its single axon to target cells. We will discuss synaptic transmission in more detail later in the chapter.

Glial cells are also important components of nervous systems

Neurons are not the only type of cell in the nervous system. In fact, there are more glial cells than neurons in the human brain. Like neurons, glial cells come in several forms and have a diversity of functions. Some glial cells physically support and orient the neurons and help them make the right contacts during their embryonic development. Other glial cells insulate axons.

In the peripheral nervous system, Schwann cells wrap around the axons of neurons, covering them with concentric layers

insulating plasma membrane (Figure 44.4). Other glial cells called oligodendrocytes perform a similar function in the central nervous system. The covering produced by Schwann cells and oligodendrocytes, called myelin, gives many parts of the nervous system a glistening white appearance. Later in the chapter we will see how the electrical insulation provided by myelin increases the speed with which axons can conduct nerve impulses.

Glial cells are well known for the many housekeeping functions they perform. Some glial cells supply neurons with nutrients; others consume foreign particles and cell debris. Glial cells also help maintain the proper ionic environment around neurons. Although they have no axons and do not generate or conduct nerve impulses, some glial cells communicate with one another electrically through a special type of contact called a gap junction, a connection that enables ions to flow between cells (see Chapter 5).

Glial cells called astrocytes (because they look like stars) contribute to the blood-brain barrier, which protects the brain from toxic chemicals in the blood. Blood vessels throughout the body are very permeable to many chemicals, including toxic ones, which would reach the brain if this special barrier did not exist. Astrocytes help form this barrier by surrounding the smallest and most permeable blood vessels in the brain.

Protection of the brain is crucial because, unlike other tissues of the body, the brain has a limited capacity to recover from damage by generating new neurons and new

neuronal connections. Throughout life, populations are, progressively

lost. Without the blood-brain barrier, the rate of neuron loss could be much greater. However, the barrier is not perfect. Since it consists of plasma membranes, it is permeable to fat-soluble substances. Anesthetics, alcohol, both of which have well-known effects on the brain, are fat-soluble chemicals.

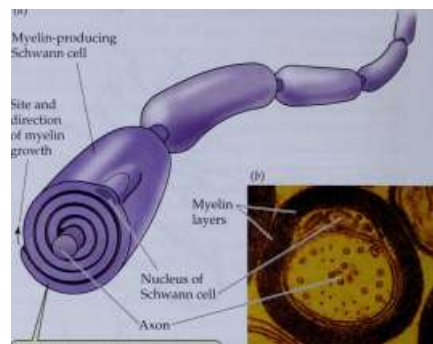
Neurons function in networks

As we learn more about the properties of neurons, it is important to keep in mind that nervous systems depend on

(a)

Myelin-producing Schwann cell

Site and direction of myelin growth



Multiple layers of plasma membrane (myelin) insulate the axon.

44.4 Wrapping Up an Axon

(a) Schwann cells wrap axons with layers of myelin, a type of plasma membrane that provides electrical insulation. (b) A group of myelinated axons, seen in cross section through an electron microscope.

neurons working together. The simplest neuronal network consists of three cells: a sensory neuron connected to a motor neuron connected to a muscle cell. Most of the neuronal networks that carry out the functions of the human nervous system are much more complex and consist of many more neurons. The human brain contains between 10^9 and 10^{11} neurons. Most of these neurons receive information from a thousand or more synapses. Thus there may be as many as 10^{14} synapses in the human brain. Therein lies the incredible ability of the brain to process information.

This astronomical number of neurons and synapses is divided into thousands of distinct but interacting networks that function in parallel and accomplish the many different tasks of the nervous system. But before we can understand how even one of these circuits works, we must understand the properties of individual neurons.

Neurons: Generating and Conducting Nerve Impulses

In this section, we explore the electrical properties of cells. After reviewing some basic electrical concepts, we examine in detail the roles of ions, ion pumps, and ion channels in establishing and altering the electrical properties of neurons. These electrical changes generate action potentials, the language by which the nervous system processes and communicates information.

The interior of cells is electrically negative in comparison to the outside. The difference in voltage across the plasma membrane of a neuron is called its membrane potential. In an unstimulated neuron, this voltage difference is called

a resting potential!

Membrane potentials can be measured with electrodes. An electrode can be made from a glass pipette pulled to a very sharp tip and filled with a solution containing ions that conduct electric charge. Using such electrodes, we can record very tiny local electrical events that occur across plasma membranes. If a pair of electrodes is placed one on each side of the plasma membrane of a resting axon, they measure a voltage difference of about -70 mV—the resting potential (Figure 44.5).

The resting potential provides a means for neurons to respond to a stimulus. A neuron is sensitive to any chemical or physical factor that causes a change in the resting potential across a portion of its plasma membrane. The most extreme change in membrane potential is a nerve impulse, which is a sudden and rapid reversal in the voltage across a portion of the plasma membrane. For a brief moment—1 or 2 milliseconds—the inside of a part of the cell becomes more positive than the outside. Nerve impulses are also called action potentials, a name that conveys their contrast with the resting potential. An action potential can travel along the plasma membrane from one part of a neuron to its farthest extensions.

Simple electrical concepts underlie neuronal functions

To understand how resting potentials are created, how they are perturbed, and how action potentials are generated and conducted along plasma membranes, it is necessary to know a little about electricity, ions, and the special ion channel proteins in the plasma membranes of neurons.

Voltage (potential) is the tendency for electrically charged particles like electrons or ions to move between two points. Voltage is to the flow of electrically charged particles what pressure is to the flow of water. If the negative and the positive poles of a battery are connected by a copper wire, electrons flow from negative to positive because there is a voltage difference between them. This flow of electrons is an electric current, and it can be used to do work, just as a current of water can be used to do work such as turning a turbine.

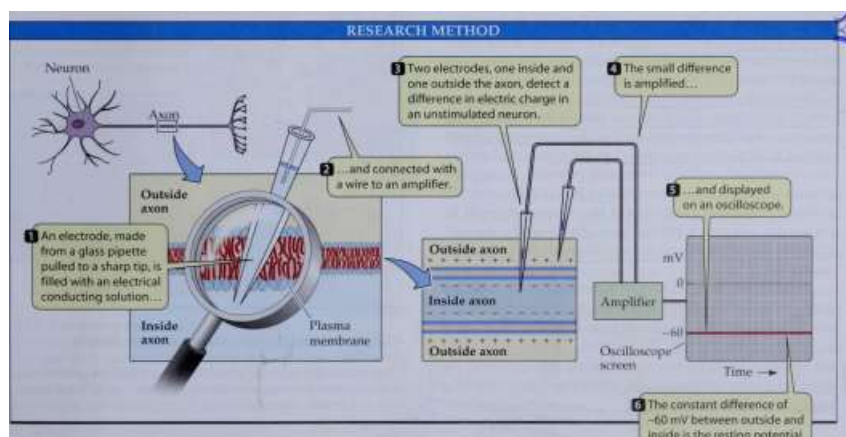
Electric charges move across cell membranes not as electrons, but as charged ions. The major ions that carry electric charges across the plasma membranes of neurons are sodium (Na^+), chloride (Cl^-), potassium (K^+), and calcium (Ca^{2+}). It is also important to remember that ions with opposite charges attract each other. With these basics of bioelectricity in mind, we can ask how the resting potential of the neuronal plasma membrane is created, and how the flow of ions through membrane channels is turned on and off to generate action potentials.

Ion pumps and channels generate resting and action potentials

The plasma membranes of neurons, like those of all other cells, are lipid bilayers that are impermeable to ions. However, these impermeable lipid bilayers contain many protein molecules that serve as ion channels and ion pumps (see Chapter 5). Ion pumps and channels are responsible for resting and action potentials.

44.5 Measuring the Resting Potential

The difference in electric charge across the plasma membrane of a neuron can be measured using two electrodes, one inside and one outside the cell. In an unstimulated neuron, this difference is constant (about -60 mV), and is known as the resting potential.



*

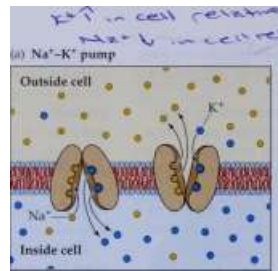
778 CHAPTER FORTY-FOUR

Ion pumps use energy to move ions or other molecules against their concentration gradients. The major ion pump in neuronal membranes is the sodium-potassium pump. The action of this pump expels Na^+ ions from the cell, exchanging them for K^+ ions from outside the cell (Figure 44.6a). The sodium-potassium pump keeps the concentration of K^+ inside the cell greater than that of the external medium, and the concentration of Na^+ inside the cell less than that of the external medium. The concentration differences established by the pump mean that K^+ would diffuse out of the cell and Na^+ would diffuse in if the ions could cross the lipid bilayer.

Ion channels are pores formed by proteins in the lipid bilayer. These water-filled pores allow ions to pass through, but they cannot actively transport ions as ion pumps do (Figure 44.6b). They are generally selective; that is, some types of ions pass

through them more easily than others. Thus there are potassium channels, sodium channels, chloride channels, and calcium channels, and there are different kinds of each. Ions move through channels by diffusion, and therefore they can move in either direction. The direction and magnitude of net movement of ions through a channel depends on the concentration gradient of that ion type across the plasma membrane.

Many ion channels in the plasma membranes of neurons behave as if they contain a "gate" that opens to allow ions to pass under some conditions, but closes under other conditions. Voltage-gated channels open or close in response to a change in the voltage across the plasma membrane. Gated channels open or close depending on



Na⁺



(b) Na⁺ and K⁺ channels

K⁺ channel

Na⁺

Na⁺

Na⁺

Na⁺

Na⁺

Na⁺

Na⁺ Na⁺ channel

Na⁺ /



The Na⁺-K⁺ pump moves Na⁺ and K⁺ ions against their concentration gradients which would otherwise run down because...

...K⁺ and Na⁺ ions tend to leak down their concentration gradients through ion-specific channels.

The presence or absence of a specific chemical that binds to the channel protein, or to a separate receptor that in turn alters the channel protein. Both voltage-gated and chemically gated channels play important roles in neuronal function.

Potassium channels are the most common open channels in the plasma membranes of resting (non-stimulated) neurons. As a consequence, resting neurons are more permeable to K⁺ than to any other ion. As Figure 44.7 shows, this characteristic explains the resting potential. Because the plasma membrane is permeable to K⁺, and because the sodium-potassium pump keeps the concentration of K⁺ inside the cell much higher than that outside the cell, K⁺ tends to diffuse out of the cell through the potassium channels.

As positively charged K⁺ ions diffuse out of the cell, they leave unbalanced negative charges behind, generating a membrane potential that tends to pull positively charged K⁺ ions back into the cell. The membrane potential at which the tendency of the K⁺ ions to diffuse out is balanced by the negative electric potential pulling them back in is called the potassium equilibrium potential.

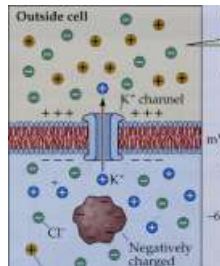
44.6 Ion Pumps and Channels

(a) The sodium-potassium pump actively moves K⁺ ions to the inside of a neuron and Na⁺ ions to the outside. (6) Channels allow ions to diffuse down their concentration gradient when they are open; thus K⁺ ions tend to leave neurons and Na⁺ ions tend to enter neurons through ion channels. The sodium channels shown here are gated, and one is closed.

The value of the potassium equilibrium potential can be calculated from the concentrations of K⁺ on the two sides of the membrane using an equation called the Nernst Equation, which is derived from the laws of physical chemistry (Figure 44.8).

In general, the resting potential is a bit less negative than this potential predicts, because resting neurons are also slightly permeable to other ions, for example Na^+ and Cl^- .

Outside cell



The tendency of K^+ ions to diffuse out leaves an excess of negative charges inside the cell, creating the resting potential.

Na^+ Inside cell

5 Negatively charged protein q

*» (5 minus charges)

Oscilloscope screen

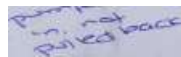
Resting potential

0 12 3

Milliseconds

44.7 Open Potassium Channels Create the Resting Potential

Open potassium channels allow K^+ ions to diffuse out of the cell, leaving unbalanced negative charges behind (mostly on chloride ions and protein molecules).



research method

Q K^+ ions diffuse out of the neuron, creating a negative potential across the plasma membrane.

t

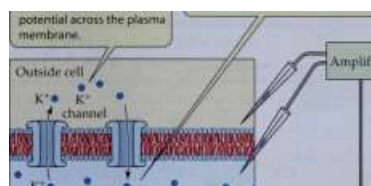
The resulting negative potential tends to pull K^+ back into the cell.

Outside cell •

$\text{K}^+ \gg \text{K}^+$ 4 channel

Amplifier

Inside cell •



The diffusional force causing K^+ to leave the neuron is $[K + L_{\text{in}}]$,

/?rin

$[K + L_{\text{in}}]$,

f

The resulting electrical force is $E_z F$.

R = universal gas constant T - absolute temperature E = voltage difference across membrane zF = number of electrical charges carried by a mole of K^+

In

\ln natural logarithm of the ratio

J_{out}

$[K^+]_{in}$

$[K^+]_{out}$: of K^+ concentrations on the two sides of the membrane

Deriving the Nernst equation:

At equilibrium the electrical force equals

the diffusional force, or

$$EzF = RT \ln \frac{[K^+]_{out}}{[K^+]_{in}}$$

$[K^+]_{in}$

J

$[K^+]_{out}$

The equilibrium potential is the membrane potential that counteracts the tendency of K^+ ions to diffuse out.

Rearranging this equality, we get an expression of the Nernst equation for the potassium equilibrium potential:

The equation can be made simpler by combining the constants, assuming the temperature is 20°C , and converting the natural logarithm to base 10 logarithm. Doing this, we get:

$$E_K = (58\text{mV}) \log \left(\frac{[K^+]_{out}}{[K^+]_{in}} \right)$$

mV

-84

Oscilloscope screen

Resting potential

Time

Applying the Nernst equation:

The concentration of K^+ ions inside a mammalian neuron is about 140 mM; outside the neuron the concentration is about 5 mM. Putting these numbers into the Nernst equation gives us a predicted resting potential of about -84 mV.

44.8 The Nernst Equation

The Nernst equation calculates membrane potential when only one type of ion can cross a membrane that separates solutions with different concentrations of that ion. The resting neuron comes close to that situation, since its permeability to K^+ ions is high and its permeability to all other ions is low.

$\frac{[K^+]_{out}}{[K^+]_{in}}$

Ion channels can alter membrane potential

Changes in the gated channels may perturb the resting potential. Imagine what would happen, for example, if Na^+ channels in the plasma membrane opened. Na^+ ions would diffuse into the cell because of their higher concentration on the outside, and they would also be attracted into the cell by the negative membrane potential. As a result of the entry of Na^+ ions, the inside of the cell would tend to become less negative. When the inside of a neuron becomes less negative in comparison to its resting condition, its plasma membrane is said to be depolarized (Figure 44.9a).

An opposite change in the resting potential would occur if gated Cl^- channels opened. The concentration of Cl^- ions is normally greater in the fluid than inside the neuron. This difference is large enough so that in many neurons, the opening of Cl^- channels causes Cl^- to enter the cell, even though the membrane potential is negative. The entry of negative charges causes the membrane potential to become even more negative. When the inside of a neuron becomes more negative in comparison to its resting condition, its plasma membrane is said to be hyperpolarized (Figure 44.9b).

The opening and closing of ion channels, which result in changes in the polarity of the plasma membrane, are the basic mechanisms by which neurons respond to electrical, chemical, or other stimuli, such as touch, sound, and light. How does a neuron use a change in its resting membrane potential to process and transmit information?

A change in resting potential may result from input at a synapse. This input, however, is a very local event that affects only

a small patch of plasma membrane. How can that information be passed to other parts of the cell? A local perturbation of membrane potential causes a flow of electrically charged ions, which tends to spread the change in membrane potential to adjacent regions of the membrane. This flow of electrically charged ions is an electric current. For example, if positively charged Na^+ ions enter the cell through sodium channels at one location, that positively charged area on the inside of the membrane attracts negative charges from surrounding areas, and thus there is a flow of electric current. However, this local flow of electric current does not spread very far before it diminishes and disappears.

*~The reason why electric currents do not travel very far in cell membranes is that these membranes are permeable to ions. An electric current traveling along a membrane is like water flowing through a leaky hose. Communication of a stimulus by the flow of electric current along plasma membranes is useful over only very short distances. Therefore, axons (the long processes of neurons) do not transmit information as a continuous flow of electric current (as telephone wires do). However, the local flow of electric current is an important part of the mechanism that generates the signals that axons do transmit over long distances: action potentials.

780 CHAPTER FORTY-FOUR

Na^+ channel

Cl^- channel

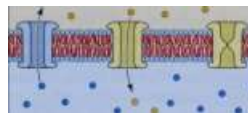
(K^+ channel)

Na^+ channel \ voltage gate open

Voltage

gate

closed



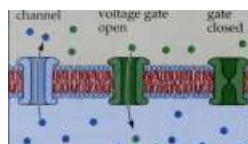
Open K^+

Cl^- channel voltage gate open

Voltage

gate

closed



_

-50 -

e^+

- e^- -70

_Gated Na^+

channel open

. Open K^+

channel

Na^+ flowing into the cell depolarizes it.

*^ synaptic input to some part of a neuron causes the plasma membrane of its cell body to depolarize, that depolarization can spread to the base of the axon, where there are voltage-gated sodium channels. When the plasma membrane containing these channels is depolarized to their threshold potential (about 5 to 10 mV more positive than the resting potential), they open briefly—for less than a millisecond.

^~^ and. The Na^+ concentration is much

.Gated Or K^+ channel open

. Open K^+ channel

V_{rest}

V_{th}

More K^+ flowing into the cell hyperpolarizes it

The resting potential is produced by open K^+ channels.

Time

44.9 Membranes Can Be Depolarized or Hyperpolarized

The resting potential created by the diffusion of K^+ out of the cell can change (see Figure 44.7). A shift to a less negative membrane potential, as occurs when a gated sodium channel opens, is called depolarization. Hyperpolarization occurs when the membrane potential becomes more negative, as when more Cl^- enters the cell through a gated channel.

Sudden changes in ion channels generate action potentials

An action potential is a sudden and major change in membrane potential that lasts for only 1 or 2 milliseconds. Action potentials are conducted along the axon of a neuron at speeds of up to 100 meters per second, which is equivalent to running the length of a football field in 1 second. How is an action potential generated, and how does it move down an axon?

If we place the tips of a pair of electrodes on either side of the plasma membrane of a resting axon and measure the voltage difference, the reading is about 60 mV, as we saw in Figure 44.5. If these electrodes are in place when an action potential travels down the axon, they register a rapid change in membrane potential, from -60 mV to +30 mV. The membrane potential rapidly returns to its resting level of -60 mV as the action potential passes.

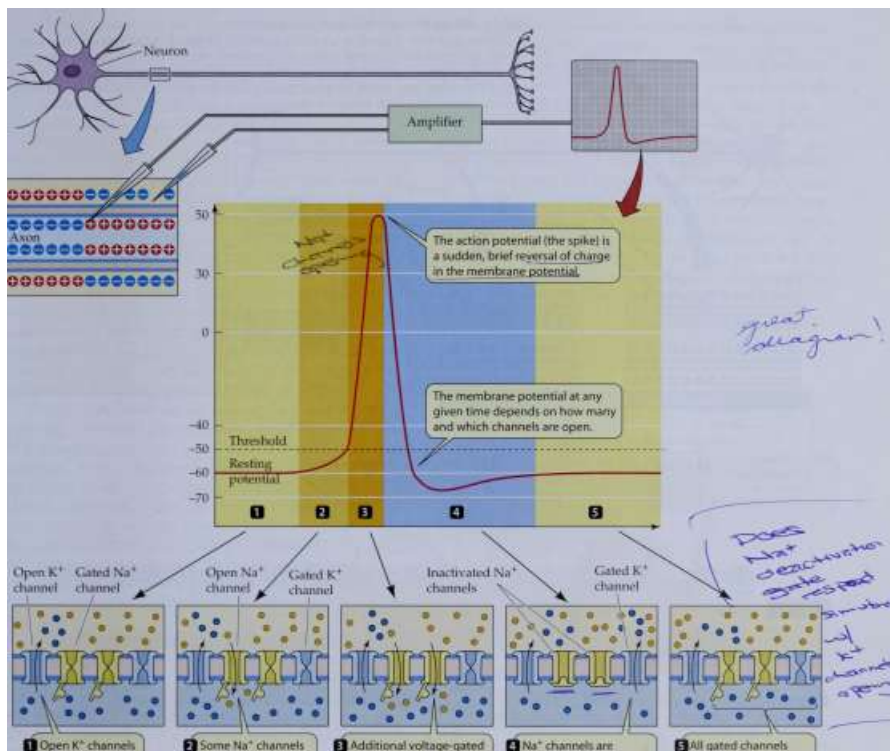
Voltage-gated sodium channels in the plasma membrane of the axon are primarily responsible for action potentials. At the normal membrane resting potential, most of these channels are closed. They are called voltage-gated channels because there is a particular membrane potential, or threshold potential, that causes them to open. For example, if

higher outside the axon than inside, so when the sodium channels open, Na^+ ions from the outside rush into the axon. The entering Na^+ makes the inside of the plasma membrane electrically positive. The opening of the sodium channels causes the rising phase of the action potential, which neurobiologists call the spike (Figure 44.10).

What causes the depolarized axon to return to resting potential? The main reason is the rapid closing of the sodium channels. Some axons also have voltage-gated potassium channels. These channels open more slowly than the sodium channels and they stay open longer, allowing K^+ to carry excess positive charges out of the axon. As a result, they help return the voltage across the membrane to its resting level.

Another feature of the voltage-gated sodium channels is that once they open and close, they can be triggered again after a short delay of 1 to 2 milliseconds. This property can be explained by the assumption that they have two voltage-sensitive gates, an activation gate and an inactivation gate (see Figure 44.10). Under resting conditions, the activation gate is closed and the inactivation gate is open. Depolarization of the membrane to threshold causes both gates to change state, but the activation gate responds faster. As a result, the channel is open for the passage of Na^+ ions for a brief time between the opening of the activation gate and the closing of the inactivation gate. The inactivation gate remains closed for 1-2 milliseconds before it spontaneously opens again, thus explaining why the membrane has a refractory period (a period during which it cannot act) before it can fire another action potential. When the inactivation gate finally opens, the activation gate is closed, and the membrane is poised to respond once again to a depolarizing stimulus by firing another action potential.

The difference in concentration of Na^+ across the plasma membrane of neurons is the driving force that drives the action potential. How rapidly does the battery run down? It might seem that a substantial number of Na^+ and K^+ ions would have to cross the membrane for the membrane potential to change from -60 mV to +30 mV and back to -60 mV again.



OOOOOOOQ

eeeeeeoooooooo

Axon

eeeeeeoooooooo

ooooooooeeeeeee

OpenK + Gated Na + channel channel

I Open K + channels create the resting potential.

Q Some Na + channels open, depolarizing the cell to threshold

|J Additional voltage-gated Na + channels open, causing a rapid spike of depolarization—an action potential.

I Na + channels are i nactivate d; gated K + channels open, repolarizing and even hyperpolarizing the eel

QAIH gated channels close. The cell returns to its resting potential.



^ 44. 7 o The Course of an Action Potential

Action potentials result from rapid changes in voltage-gated sodium and potassium channels. Like the resting potential, they can be measured using two electrodes (see

Figure 44.5).

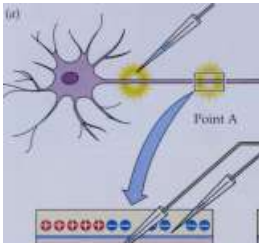
In fact, only about one Na + (or K +) ion for every 10 million present actually moves through the channels^ during the passage of an action potential. Thus the effect of a single action potential on the concentration ratios of Na + or K + is very small, and it is not difficult for the sodium-potassium pump to keep the "battery" charged, even when the cell is generating many action potentials every second.

Action potentials are conducted down axons without reduction in the signal

Action potentials can travel over long distances with no loss ofTne jignaTTfwe place two pairs ofel ectro des at two different locations along an axon, we can record an action potential at those two location s as it travels down the ax on (Figure 44.11a). The magnitude of the action potential does not change between the two recordings. The action potential is an jill-o r-nothing, self-reg enerating event.

The action potential i s all-or-riofhin g~because of the interaction between the v oltag-e-gated s odium channels and the membrane potential. If the membrane is depolarized

Electric
stimulus



Axon

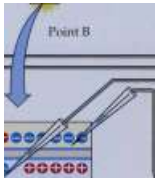
eeeeeeoooooooo
oooooooooooo

Electrode 1 (point A)

44.1 7 Action Potentials Travel along Axons

(a) There is no loss of signal as an action potential travels along an axon. (fc>) When an action potential occurs in one region of membrane, electric currents flow to adjacent areas of membrane and depolarize them. As voltage-gated channels in those areas reach threshold, they create an action potential. In this way, an action potential continuously regenerates itself along the axon.

oooooooo©



Axon

eeeeeeccooQOQQ
oooooooooooo

Electrode 2 (point B)

Amplifier

. . H Amplifier



Electrode 1



Electrode 2

Time
(b)

Electric stimulus

Q Voltage-gated Na + channels open in response to the electric stimulus, generating an action potential.

Axon
n

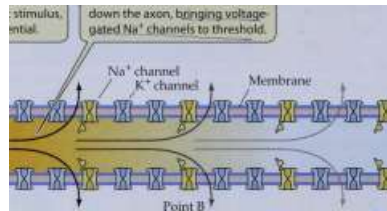


n11111

Na⁺ o^o

IA depolarizing current spreads down the axon, bringing voltage-gated Na⁺ channels to threshold.

Point A



11 w m m

Point C

f

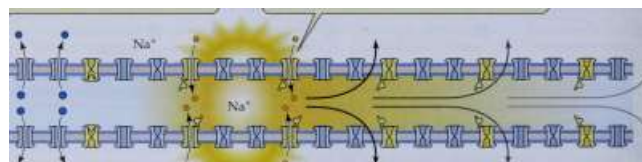
Upstream Na⁺ channels inactivate, making the membrane refractory. Voltage-gated K⁺ channels open, repolarizing the axon.

Q Spreading depolarization causes neighboring Na⁺ channels to open, renewing the action potential.

K⁺ <

I 1 1 1

Time = 1



A i i k i d s e h I f m m f n m k m m m m i l n

K⁺ <

* Point A*

K⁺

o Point B

• •

Point C

f

The process is repeated, propagating the action potential along the axon.

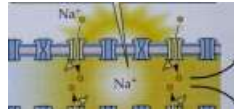
m m :p m i j s m m j h p i | M p f s i

• K* •

Point A

m h ■ i ® i f i

K⁺ / J Point B \ \



nm if m m if i

Point C ° j \ | a +

slightly and approaches the threshold potential for voltage-gated sodium channels, some of them open. Some Na⁺ ions cross the plasma membrane and depolarize it even more, bringing more voltage-gated sodium channels to threshold, and so on. This positive feedback mechanism ensures that action potentials always rise to their maximum value.

The action potential is self-regenerating because it spreads by current flow to adjacent regions of the membrane.

The resulting depolarization brings those areas of plasma membrane to the threshold potential for the voltage-gated Na⁺ channels. So when an action potential occurs at one location on an axon, it stimulates the adjacent region of axon to generate an action potential, and so on down the length of the axon (Figure 44.11b).

We can initiate an action potential by shocking an axon with an electric current delivered through a stimulating electrode that depolarizes the membrane enough to reach threshold. Now we can observe the changes in membrane potential associated with the passage of that action potential past the recording electrodes (Figure 44.11b).

Positive ions flood into the axon at the site of the action potential. Once inside, positive ions spread by current flow to adjacent regions of the axon, making those regions less negative. As this depolarization of the adjacent plasma membrane brings it to the threshold potential, an action potential is generated there. Because an action potential always brings the adjacent area of membrane to threshold, the action potential propagates itself along the axon. The action potential is self-regenerating and propagates itself in both directions; however, it cannot reverse itself, because the part of the membrane it came from is in its refractory period.

Action potentials do not travel along all axons at the same speed. They travel faster in large-diameter axons than in small-diameter axons. In invertebrates, the axon diameter determines the rate of conduction, and axons that transmit messages involved in escape behavior are very thick. The giant axons that enable squid to escape predators are almost 1 mm in diameter.

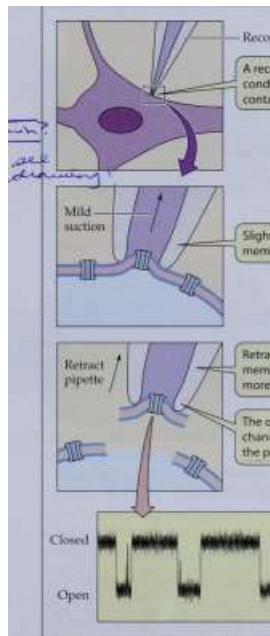
Ion channels and their properties can be studied directly

The size of the squid giant axon made it possible for the British neurophysiologists A. L. Hodgkin and A. F. Huxley to study the electrical properties of axonal membranes 60 years ago. They used thin electrodes to measure voltage differences across the plasma membrane of the squid giant axon and to pass electric current into the axon to change its resting potential. They also changed the concentrations of Na⁺ and K⁺ ions both inside and outside the axon and measured the changes in resting and action potentials.

On the basis of their many careful experiments, Hodgkin and Huxley developed the story we have discussed so far. However, they were working long before technology enabled the actual demonstration of ion channels. Hodgkin and Huxley could only hypothesize the existence of ion channels and their properties.

With current techniques, neurobiologists can record currents caused by the openings and closings of single ion channels directly. Patch clamping, developed in the 1980s by Bert Sakmann and Erwin Neher, made possible the study of single ion channels in plasma membranes. In patch clamping, a glass pipette with a tip only 1 or 2 microns in diameter is placed in contact with the plasma membrane (Figure 44.12). Slight suction makes a seal between the pipette and the patch of membrane under its tip. Retracting the pipette can detach a bit of plasma membrane. Movements of ions, and therefore electric charges, through channels in the patch of membrane can be recorded through the pipette. Frequently, a patch will contain only one or a few ion channels; thus the electrical recording from that patch can show individual channels opening and closing.

RESEARCH METHOD



Recording pipette

A recording pipette filled with a conducting solution is placed in contact with a neuron's membrane.

Slight suction clamps a patch of the membrane to the pipette tip.

Retracting the pipette removes the membrane patch, often with one or more ion channels in it.

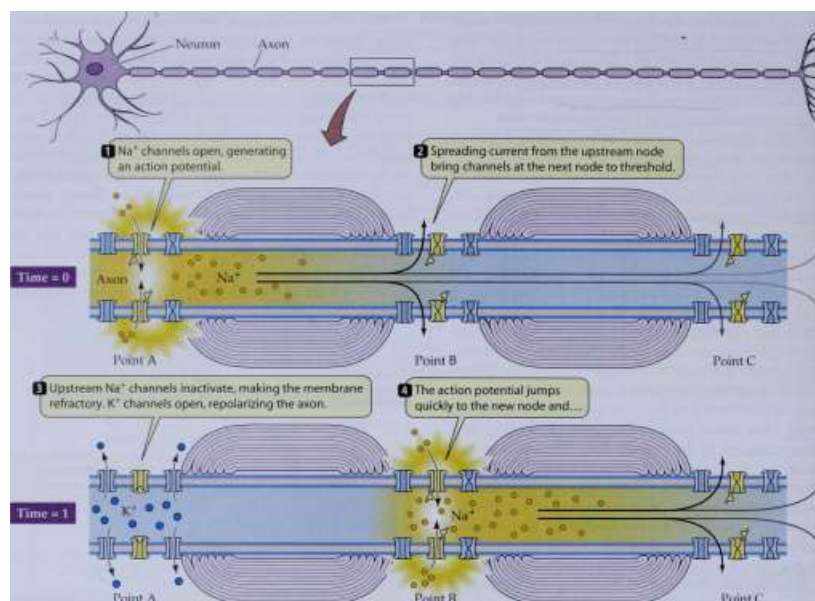
The opening and closing of ion channels can be recorded through the pipette.



44.12 Patch Clamping

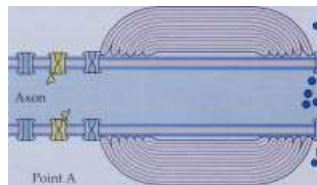
A patch clamp electrode can record the opening and closing of a single ion channel.

784 CHAPTER FORTY-FOUR



Point A

Point C



f

...continues from node to node.

I ffl II

. • f • " •

Point A

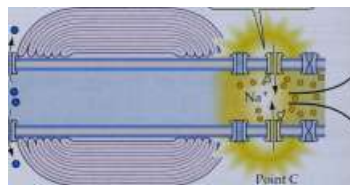
44.13 Saltatory Action Potentials

Action potentials appear to jump from node to node in myelinated axons.

Action potentials can jump down axons

In nervous systems that are more complex than those of invertebrates, increasing the speed of action potentials by increasing the diameter of axons is not feasible because of the huge number of axons that are required. Each of our eyes, for example, has about a million axons extending from it. Evolution has increased propagation velocity in vertebrate axons in a way that does not require large size.

Point B



Point C

When we discussed glial cells earlier in the chapter, we saw that glial cells of one type, called Schwann cells, send out projections that wrap around axons, covering them with concentric layers of plasma membrane (see Figure 44.4). These myelin wrappings are not continuous, but have regularly spaced gaps called nodes of Ranvier where the axon is not covered (Figure 44.13).

Myelin electrically insulates the axon; that is, charged ions cannot cross the regions of the plasma membrane that are wrapped in myelin. Additionally, ion channels are clustered at the nodes. Thus an axon can fire an action potential

only at a node of Ranvier, but that action potential cannot be conducted through the adjacent patch of axon covered with myelin. The positive charges that flow into the axon at the node, however, spread down the axon. When the spread of current causes the plasma membrane at the next node to depolarize to threshold, an action potential is fired at that node. Action potentials therefore appear to jump down the axon.

The speed of conduction is increased in these myelin-wrapped axons because electric current flows very fast through the cytoplasm in comparison to the time required for channels to open and close. This form of impulse propagation is called saltatory (jumping) conduction and is much quicker than continuous propagation of action potentials down an unmyelinated axon.

Neurons, Synapses, and Communication

The most remarkable abilities of nervous systems stem from interactions among connected neurons. These interactions process and integrate information. Our nervous systems can orchestrate complex behaviors, deal with complex concepts, and learn and remember because large numbers of neurons interact with one another. The mechanisms of these interactions depend on synapses between cells. Synapses are structurally specialized junctions where one cell influences another cell directly through the transfer of an electrical or chemical message. The cell that sends the message is called the presynaptic cell, and the cell that receives it is called the postsynaptic cell. The most common type of synapse in the nervous system is the chemical synapse—one in which chemical messages released by a presynaptic cell induce changes in a postsynaptic cell.

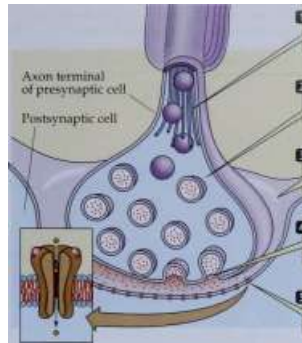
In this section, we examine the specializations and functions of presynaptic and postsynaptic cells. We discover how synapses can integrate information, and at the end of the section we examine the diversity of chemical messages released by neurons.

The neuromuscular junction is a classic chemical synapse

Synapses between motor neurons and muscle cells are called neuromuscular junctions and they are excellent models for

how chemical synaptic transmission works. Like other neurons, the motor neuron that innervates a muscle has only one axon, but that axon can have many branches, each with an axon terminal that forms a neuromuscular junction with a muscle cell. At each axon terminal is an enlarged knob or buttonlike structure that contains many spherical vesicles filled with chemical messenger molecules, or neurotransmitters (Figure 44.14). The neurotransmitter used by all neurons that innervate vertebrate skeletal muscle is acetylcholine. The portion of the axon terminal plasma membrane that forms a synapse with a

Axon terminal of presynaptic cell



Enzymes for neurotransmitter synthesis are transported along microtubules.

Neurotransmitter is synthesized in the axon terminal and packaged in the vesicles.

The presynaptic and postsynaptic membranes are separated by the synaptic cleft.

Neurotransmitter is released into the synaptic cleft from the presynaptic cell.

Neurotransmitter binds to the receptor and opens the channel.

44.14 The Neuromuscular Junction Is a Chemical Synapse

A motor neuron communicates chemically with a muscle cell at a neuromuscular junction when a neurotransmitter called acetylcholine (red dots) crosses the synaptic cleft.

muscle cell is called the presynaptic membrane. Acetylcholine is released by exocytosis when the membrane of a vesicle fuses with the presynaptic membrane.

Where does the neurotransmitter come from? Some neurotransmitters, like acetylcholine, are synthesized in the axon terminal and packaged in vesicles. The enzymes required for acetylcholine biosynthesis, however, are produced in the cell body of the motor neuron and are transported down the axon to the terminals along microtubules. Other kinds of neurotransmitters, such as peptide neurotransmitters, are produced in the cell body and transported down the axon to the terminals.

transmitters, are produced in the cell body and transported down the axon to the terminals.

the postsynaptic

membrane

If the neuromuscular junction is a modified part of the muscle cell plasma membrane called a motor end plate. The space between the presynaptic membrane and the postsynaptic membrane is the synaptic cleft. In chemical synapses, the synaptic cleft is, on average, about 200 nm wide, and the neurotransmitter released into the cleft diffuses across to the postsynaptic membrane (see Figure 44.14).

The patches of muscle cell plasma membrane that form a motor end plate contain acetylcholine receptor molecules. These receptors are chemically gated channels that allow both Na^+ and K^+ to pass through. Since the resting membrane is already permeable to K^+ , the major change that occurs when these channels open is the movement of Na^+ into the cell. When a receptor binds acetylcholine, its channel opens, and Na^+ moves across the membrane, depolarizing the motor end plate (Figure 44.15).

Acetylcholine action is limited by the enzyme, acetylcholinesterase, found in the synaptic cleft. This powerful enzyme cleaves any acetylcholine molecules it encounters.

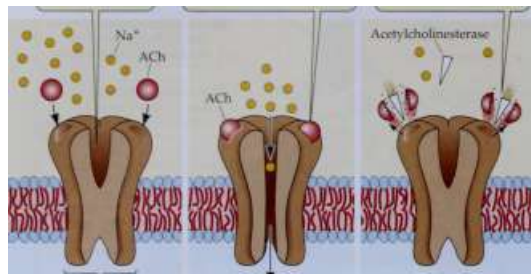
:

786 CHAPTER FORTY-FOUR

The acetylcholine receptor-mediated channel is normally closed.

When ACh binds at specific sites on the receptor the channel opens, allowing Na^+ to enter the postsynaptic cell.

Acetylcholinesterase breaks down ACh, causing the receptor-mediated channel to close.



ACh receptor

T

Postsynaptic cell depolarizes

Thus, the activity of the neuromuscular junction is a balance between the release of acetylcholine by the presynaptic membrane and its destruction by acetylcholinesterase in the synaptic cleft. The breakdown products of neurotransmitter degradation are taken up by the presynaptic terminal and resynthesized into acetylcholine.

The arrival of an action potential causes the release of neurotransmitter

Neurotransmitter is released from the presynaptic membrane when an action potential arrives at the axon terminal. The presynaptic membrane has a type of voltage-gated ion channel found nowhere else on the axon: a voltage-gated calcium channel. When the action potential reaches the axon terminal, it causes these calcium channels to open (Figure 44.16). Because Ca^{2+} concentration is greater outside the cell than inside the cell, Ca^{2+} rushes in.

The increase in Ca^{2+} inside the presynaptic cell causes the vesicles containing acetylcholine to fuse with the presynaptic membrane and empty their contents into the synaptic cleft. The acetylcholine molecules diffuse across the cleft and bind to the receptors on the motor end plate, causing the sodium channels to open briefly and depolarize it.

The postsynaptic membrane integrates synaptic input

The postsynaptic membranes of neuromuscular junctions differ from the presynaptic membranes in an important way. Motor end plates have very few voltage-gated sodium channels; therefore, they do not fire action potentials. This is true not only of motor end plates, but also of dendrites and of most regions of neuronal cell bodies. The binding of neurotransmitter to receptors at the motor end plate and the resultant opening of chemically gated ion channels per-

44.15 The Actions of Acetylcholine and Acetylcholinesterase Balance Out

When a receptor on the motor end plate binds acetylcholine (ACh), a chemically gated channel opens, and Na^+ ions move into the postsynaptic cell, making the cell more positive inside (depolarization). Acetylcholinesterase in the synapse destroys ACh, closing the channel; the breakdown products are then resynthesized into more ACh.

turb the resting potential of the postsynaptic membrane. This local change in membrane potential spreads to neighboring regions of the plasma membrane of the postsynaptic cell.

Eventually, the spreading depolarization may reach an area of membrane that does

com

tage-gated channels. The entire

plasma membrane of a skeletal muscle fiber, except for the motor end plates, has voltage-gated sodium channels. If the axon terminal of a motor neuron releases sufficient amounts of neurotransmitter to depolarize a motor end plate enough to bring the surrounding membrane to threshold, an action potential is fired. This action potential is then conducted throughout the muscle cell's system of membranes, causing the cell to contract. (We'll learn more about muscle membrane action potentials and the contraction of muscle cells in Chapter 47.)

How much neurotransmitter is enough? Neither a single acetylcholine molecule nor the contents of an entire vesicle (about 10,000 acetylcholine molecules) are enough to bring the plasma membrane of a muscle cell to threshold. However, a single action potential in an axon terminal releases about 100 vesicles, which is enough to fire an action potential in the muscle cell and cause it to contract.

Synapses between neurons can be excitatory or inhibitory

In vertebrates, the synapses between motor neurons and skeletal muscle are always excitatory; that is, motor end

plates always respond to acetylcholine by depolarizing the postsynaptic membrane. However, synapses between neurons can be either excitatory or inhibitory.

Recall that a neuron may have many dendrites. Axon terminals from many neurons may make synapses with those dendrites and with the cell body. The axon terminals of different presynaptic neurons may store and release different neurotransmitters, and membranes of the dendrites and cell body of a postsynaptic neuron may have receptors for a variety

of neurotransmitters. Thus a postsynaptic neuron can receive various chemical messages. If the postsynaptic neuron's response to a neurotransmitter is depolarization, as at the neuromuscular junction, the synapse is

Nerve impulse

NEURONS AND NERVOUS SYSTEMS 787

f

An action potential arrives and initiates synaptic transmission.

t

Na⁺ channels open, depolarizing the axon terminal membrane.



Depolarization of the terminal membrane causes voltage-gated Ca²⁺ channels to open.

Ca²⁺ enters the cell and triggers fusion of neurotransmitter vesicles with the presynaptic membrane.

Action potential

1. Neurotransmitter molecules diffuse across the synaptic cleft and bind to receptors on the postsynaptic membrane.

2. Activated receptors open chemically gated Na⁺ channels and depolarize the postsynaptic membrane. The spreading depolarization fires an action potential in the adjacent membrane.

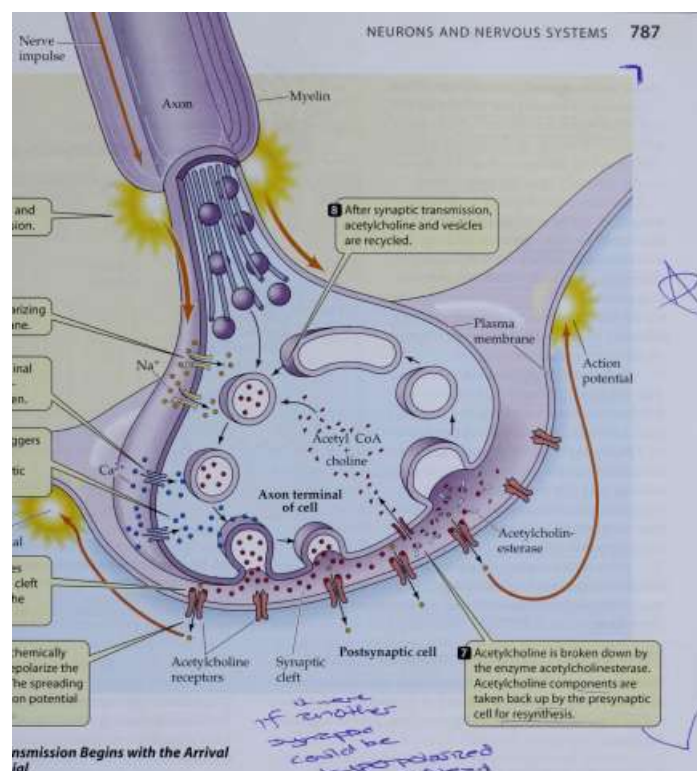


Figure 44.16 Synaptic Transmission Begins with the Arrival of an Action Potential

The figure diagrams the sequence of events involved in transmission at the motor end plate, a typical chemical synapse.

If the response is hyperpolarization, the synapse is inhibitory (see Figure 44.9).

How do inhibitory synapses work? In vertebrates, the two most common inhibitory neurotransmitters are γ -amino butyric acid (GABA) and glycine. The postsynaptic cells at inhibitory-synapses have chemically gated chloride channels. When these channels are activated by a neurotransmitter, they can hyperpolarize the postsynaptic membrane. Thus the release of neurotransmitter at an inhibitory synapse makes the postsynaptic cell less likely to fire an action potential.

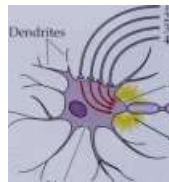
Neurotransmitters that depolarize the postsynaptic membrane are excitatory; they bring about an excitatory postsynaptic potential (EPSP). Neurotransmitters that hyperpolarize the postsynaptic membrane are inhibitory; they bring about

an inhibitory postsynaptic potential (IPSP).

The postsynaptic membrane sums excitatory and inhibitory input

Individual neurons "decide" whether or not to fire an action potential by summing excitatory and inhibitory post-synaptic potentials. This summation ability of neurons is the major mechanism by which the nervous system integrates information. Each neuron may receive 1,000 or more synaptic inputs, but it has only one output: an action potential in a single axon. All the information contained in the thousands of inputs a neuron receives is reduced to the rate at which that neuron generates action potentials in its axon. For most neurons, the area for "decision making" is the axon hillock, the region of the cell body at the base of the axon. The plasma membrane of the axon hillock is not insulated by glial cells and has many voltage-gated channels. Excitatory and inhibitory post synaptic potentials from synapses anywhere on the dendrites or the cell body spread to the axon hillock by current flow. If the resulting combined ΔV potential depolarizes the axon hillock to threshold, the axon fires an action potential. Because postsynaptic potentials de-

Dendrites



Neuron

(«)

>

c

01

o

C

+ 60

Resting potential

788 CHAPTER FORTY-FOUR

crease as they spread from the site of the synapse, all postsynaptic potentials do not have equal influences on the axon hillock. A synapse at the end of a dendrite has less influence than a synapse on the cell body near the axon hillock.

Excitatory and inhibitory postsynaptic potentials can be summed over space or over time. ΔV summation adds up the simultaneous influences of synapses at different sites on the postsynaptic cell (Figure 44.17a). Temporal summation adds up postsynaptic potentials generated at the same site in a rapid sequence (Figure 44.17b).

All the neuron-to-neuron synapses that we have discussed up to now are between the axon terminals of a presynaptic cell and the cell body or dendrites of a postsynaptic cell. Synapses can also form between the axon terminals of one neuron and the axon terminals of another neuron. Such a synapse can modulate how much neurotransmitter the second neuron releases in response to action potentials traveling down its axon. We refer to this mechanism of regulating synaptic strength as presynaptic excitation/inhibition.

There are two types of neurotransmitter receptors

Most neurotransmitter receptors induce changes in postsynaptic cells by opening or closing ion channels. How they do so is the basis for grouping receptors into two general families.

► Ionotropic receptors are themselves ion channels. Ionotropic neurotransmitter binding by an ionotropic receptor causes a direct change in ion movements across the postsynaptic cell membrane.

► Metabotropic receptors are not ion channels, but they induce changes in the postsynaptic cell that can secondarily lead to changes in ion channels.

Postsynaptic cell responses mediated by metabotropic receptors are generally slower and longer-lasting than those induced by ionotropic receptors.

The acetylcholine (ACh) receptor of the motor end plate of muscle cells is an example of an ionotropic receptor. This receptor is called the nicotinic ACh receptor because it also binds the drug nicotine. The receptor consists of five sub-units, each of which extends through the plasma membrane (see Figure 44.15). When assembled, the subunits create a central pore that is the ion channel. There are several different kinds of subunits, and only one kind has the ability to bind ACh or nicotine. Each functional receptor-ion channel has two α and three β subunits.

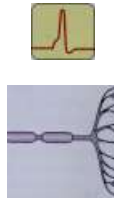
other subunits.

2 I Excitatory

3 [synapses

Axon hillock

Axon



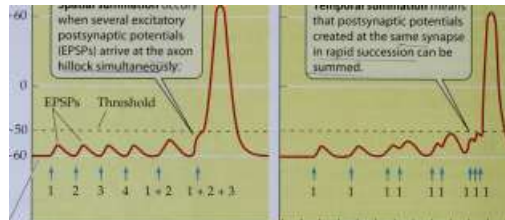
Spatial summation occurs when several excitatory postsynaptic potentials (EPSPs) arrive at the axon hillock simultaneously:

Action potential

Action potential

(b)

I



Temporal summation means that postsynaptic potentials created at the same synapse in rapid succession can be summed.

Milliseconds •

Synapse number

44.17 The Postsynaptic Membrane Sums Information

Individual neurons sum excitatory and inhibitory postsynaptic potentials over space and time. When the sum of the potentials depolarizes the axon hillock to threshold, the neuron generates an action potential.

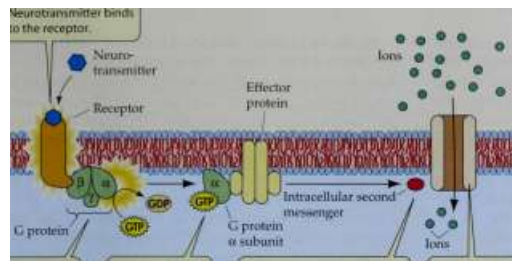
Metabotropic receptors are also transmembrane proteins, but instead of acting as ion channels, they initiate an intracellular signaling process that can result in the opening or closing of an ion channel. These receptors have seven transmembrane domains, and they are coupled to G proteins (Figure 44.18; see Chapter 15). When a neurotransmitter binds to the extracellular domain of a metabotropic receptor, the intracellular domain activates a G protein. In its inactive state, the G protein has three subunits, one of which (the α subunit) is bound to a molecule of GDP. When the receptor binds its neurotransmitter, the GDP is replaced with a GTP molecule, and the α subunit separates from the other two subunits (called β and γ). The separated subunits move across the membrane to where they can either directly activate an ion channel or activate an intermediate effector protein that eventually activates the ion channel.

An example of a metabotropic receptor is the muscarinic ACh receptor, so called because it binds both ACh and a molecule called muscarine that is a toxin in some poisonous mushrooms. Muscarinic ACh receptors are found in heart muscle. When activated, they cause the opening of potassium channels, which slows the heartbeat.

Metabotropic receptors can induce intracellular changes other than the opening or closing of ion channels. G proteins can activate a number of intracellular second messenger systems.

T

Neurotransmitter binds to the receptor.



NEURONS AND NERVOUS SYSTEMS 789

44.78 Metabotropic Receptors Act through G Proteins

Metabotropic receptors activate G proteins, which can influence ion channels directly or through second messengers.

QThe receptor

activates a

G protein.

HAG protein sub-unit activates an effector protein.

QThe activated effector protein activates an intracellular

second messenger cascade..

_ J

o ...resulting in the opening of an ion channel or other cellular responses.

terns (see Chapter 15). Through these signaling pathways, the metabotropic receptor can activate protein kinases, activate or inactivate enzymes, and alter gene expression.

Electrical synapses are fast but do not integrate information well

Electrical synapses, or gap junctions, are different from chemical synapses because they couple neurons electrically. At gap junctions, the presynaptic and postsynaptic cell membranes are separated by a space of only 2 to 3 nm, and specific membrane proteins called connexons link the two neurons by forming molecular tunnels between the two cells. Ions and small molecules can pass directly from cell to cell through the connexons (see Figure 15.19). Electrical transmission across gap junctions is very fast and can proceed in either direction; that is, stimulation of either neuron can result in an action potential in the other. In contrast, chemical synapses are slower, and they are unidirectional.

Gap junctions are less common in the complex nervous systems of vertebrates than are chemical synapses for several reasons. First, electrical continuity between neurons does not allow temporal summation of synaptic inputs, which is one way that complex nervous systems integrate

information. Second, an effective electrical synapse requires a large area of contact between the presynaptic and postsynaptic cells. This condition rules out the possibility of

thousands of synaptic inputs to a single neuron—which is the norm in complex nervous systems. Third, electrical synapses cannot be inhibitory. Finally, there appears to be little plasticity (modifiability) in electrical synapses. Thus, electrical synapses are good for rapid communication but not for processes of integration and

The action of a neurotransmitter depends on the receptor to which it binds

More than 25 neurotransmitters are now recognized, and more will surely be discovered. Table 44.1 gives some examples. Acetylcholine; as we have seen, is an important neurotransmitter because it is the means whereby nerves command skeletal muscles to contract. Acetylcholine also



plays roles in certain synapses between neurons in the central nervous system, but it accounts for only a small percentage of the total neurotransmitter content of the CNS. The workhorse neurotransmitters of the CNS are simple amino acids: glutamate (excitatory) and glycine and GABA (inhibitory).

Another important group of neurotransmitters in the CNS is the monoamines, which are derivatives of amino acids. They include dopamine and norepinephrine (derivatives of tyrosine) and serotonin (a derivative of tryptophan). Peptides also function as neurotransmitters. An exciting recent discovery revealed that two gases, carbon monoxide and nitric oxide, are used by neurons as intercellular messengers even though they do not have the characteristics of classic neurotransmitters (that is, they do not have receptors).

The complexity of neurotransmission is increased by the fact that each neurotransmitter has multiple receptor types. We have already seen that acetylcholine has two well-known receptor types, nicotinic and muscarinic, one being ionotropic and the other metabotropic. Both types of ACh receptors are found in the CNS, where nicotinic receptors tend to be excitatory

and muscarinic receptors tend to be inhibitory. ACh actions can differ outside of the CNS as well. ACh acting through nicotinic receptors causes the smooth muscle of the gut to depolarize and therefore increases its motility, but ACh acting through muscarinic receptors causes cardiac muscle to hyperpolarize and therefore decreases the contractility of the heart.

We could give many more examples of neurotransmitters that have different effects in different tissues, but the important thing to remember is that the action of a neurotransmitter depends on the receptor to which it binds.

Glutamate receptors may be involved in learning and memory

Glutamate can bind to a variety of receptors, including

metabotropic receptors that activate G proteins and ionotropic receptors that are directly linked to ion channels that open when the receptor binds glutamate. The glutamate receptors are divided into several classes because they are differentially activated by other chemicals that mimic the action of glutamate. One class of ionotropic glutamate receptor is the NMDA receptor; they can be activated by the chemical N-methyl-D-aspartate. Another class of ionotropic glutamate receptors is activated by a different chemical, abbreviated as AMPA.

790 CHAPTER FORTY-FOUR

Glutamate is an excitatory neurotransmitter, so activation of all glutamate receptors results in sodium entry into the neuron and depolarization. But the timing of the response to activation of these different types of receptors differs significantly. The AMPA receptors, for example, allow a rapid influx of Na^+ into the postsynaptic cell. The NMDA receptors allow a slower and longer-lasting influx of Na^+ . The NMDA receptors also require the cell to be somewhat depolarized through the action of other receptors before they will open channels and permit Na^+ influx. When they do respond, the channels they open also allow Ca^{2+} to enter the cell. Ca^{2+} ions act as second messengers in the cell and can trigger a variety of long-term cellular changes.

Figure 44.19 shows how the AMPA and NMDA receptors can work in concert. The critical difference is that at normal resting potential the NMDA channel is blocked by a magnesium ion (Mg^{2+}). Slight depolarization of the neuron due to other inputs displaces Mg^{2+} from the NMDA channels and allows Na^+ and Ca^{2+} to pass through the channels when they are activated by glutamate. These special properties of the NMDA receptor are probably involved in learning and memory.

Most of the synaptic events we have studied so far happen very quickly. It is therefore a special challenge to understand how the messages carried by action potentials can result in long-term events such as learning and memory. Our understanding of these processes has been greatly af-



rn

44.1

Some Well-Known Neurotransmitters

NEUROTRANSMITTER

ACTIONS

COMMENTS

Acetylcholine

Monoamines

Norepinephrine

Dopamine Histamine Serotonin

Purines ATP

Adenosine

Amino acids

Glutamate

Glycine

Gamma-aminobutyric acid (GABA)

Peptides

Endorphins

Enkephalins

Substance P

Gas

Nitric oxide

The neurotransmitter of vertebrate motor neurons and of some neural pathways in the brain

Used in certain neural pathways in the brain.

Also found in the peripheral nervous system,

where it causes gut muscles to relax and the

heart to beat faster A neurotransmitter of the central nervous

system A minor neurotransmitter in the brain

A neurotransmitter of the central nervous system that is involved in many systems, including pain control, sleep /wake control, and mood

Co-released with many neurotransmitters

Transported across cell membranes; not synaptically released

The most common excitatory neurotransmitter in the central nervous system

Common inhibitory neurotransmitters

Used by certain sensory nerves, especially in pain pathways

Widely distributed in the nervous system

Broken down in the synapse by acetylcholinesterase; blockers of this enzyme are powerful poisons

Related to epinephrine and acts at some of the same receptors

Involved in schizophrenia. Loss of dopamine

neurons is the cause of Parkinson's disease Thought to be involved in maintaining

wakefulness Certain medications that elevate mood and

counter anxiety act by increasing serotonin

levels

Large family of receptors may shape postsynaptic responses to classical neurotransmitters

Largely inhibitory effects on postsynaptic cells

Some people have reactions to the food additive monosodium glutamate because it can affect the nervous system

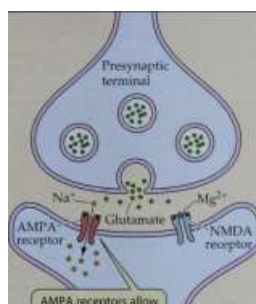
Drugs called benzodiazepines, used to reduce anxiety and produce sedation, mimic the actions of GABA

Receptors are activated by narcotic drugs: opium, morphine, heroin, codeine

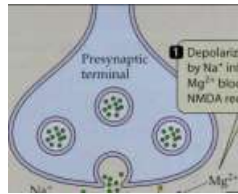
Not a classic neurotransmitter, it diffuses across membranes rather than being released synaptically. A means whereby a postsynaptic cell can influence a presynaptic cell

(a)

(b)



AMPA receptors allow rapid influx of Na^+ .



Depolarization of the cell by Na^+ influx, displaces Mg^{2+} blocking the NMDA receptor...

Na^+ ...



...which then opens to allow both Na^+ and Ca^{2+} ions.

Na^+

Ca^{2+} acts as a second messenger triggering long-term cellular change.

44.19 Two Ionotropic Glutamate Receptors

(a) AMPA receptors allow rapid influx of Na^+ into the postsynaptic cell, (b) NMDA receptors allow both Na^+ and Ca^{2+} to enter the cell.

affected by a phenomenon called long-term potentiation, or

LTP, that was discovered by neurobiologists working with slices of brain that they kept alive in dishes of culture medium. Using these brain slice preparations, it is possible to stimulate and record from specific brain regions, or even specific cells.

In the studies leading to the discovery of LTP, experimenters repeatedly stimulated the synaptic inputs to a particular neuron and observed the usual action potential response. When the neuron was stimulated repeatedly in rapid succession, however, they found that the properties of the responding neuron changed. The size of the postsynaptic response was enhanced, or potentiated, and this change lasted for days or weeks (Figure 44.20).

How does this potentiation occur? The answer in some areas of the brain now seems quite clear. With low levels of stimulation, the glutamate released by presynaptic cells activates only the AMPA receptors, and the postsynaptic cell simply responds with action potentials. With higher levels of stimulation, however, the NMDA receptor is activated, allowing both Na^+ and Ca^{2+} ions to enter the postsynaptic neuron. The Ca^{2+} ions induce long-term changes in the postsynaptic neuron that make it more sensitive to synaptic input.

Exploiting the LTP system, Dr. Joe Tsien and his students and collaborators at Princeton University have made mice smarter by increasing the ability of their NMDA receptors to induce long-term changes in synaptic transmission. The experimenters genetically engineered mice so that their NMDA receptors had a slightly altered structure and were activated for a longer time whenever they bound a molecule of glutamate. The mice with these altered NMDA re-

ceptors learned tasks better, ran mazes faster, and remembered the mazes longer than normal mice. Maybe the world does not need smarter mice, but these exciting experiments confirm that we are on the right track to understanding how the brain achieves learning and memory.

To turn off responses, synapses must be cleared of neurotransmitter

The action of neurotransmitters is as important as turning it on. If released neurotransmitter molecules simply remained in the synaptic cleft, the postsynaptic membrane would become saturated with neurotransmit-

ter, and receptors would be constantly bound. As a result, the postsynaptic cell would remain hyperpolarized and would be unresponsive to short-term changes in the presynaptic cell. The more discrete each separate neural signal is, the more information can be processed in a given time. Thus neurotransmitter must be cleared from the synaptic cleft shortly after it is released by the axon terminal.

Neurotransmitter action may be terminated in several ways. First, enzymes may destroy the neurotransmitter. As we have seen, acetylcholine is rapidly destroyed by the enzyme acetylcholinesterase, which is present in the synaptic cleft in close association with the acetylcholine receptors on the postsynaptic membrane. Some of the most deadly nerve gases that were developed for chemical warfare work by inhibiting acetylcholinesterase. As a result, acetylcholine lingers in the

synaptic clefts, causing the victim to die of spastic (contracted) muscle paralysis. Some agricultural insecticides, such as malathion, also inhibit acetylcholinesterase and can poison farm workers if used without safety precautions.

Second, neurotransmitter may simply diffuse away from the cleft. Third, neurotransmitter may be taken up via active transport.

792 CHAPTER FORTY-FOUR

EXPERIMENT

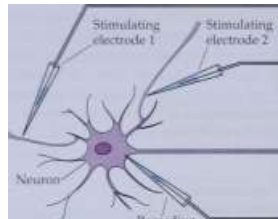
Question: Can stimulation change the property of a synapse so that it has "memory"?

METHOD Cell is alternatively stimulated through

two input pathways and responses recorded.

RESULTS

t



Recording electrode

= C

U U

> ~ ~ -

• *

c 3

'J1 x!

Response to stimulating electrode 1

• • • • •

High frequency stimulation is given through stimulating electrode 1.

"7

r.

'•'... ---•<•-

> * ^ % - * , * ^ / t * > . v * • ^ ^

Time



Q The cell responds similarly to the same, repeated level of stimulation from either electrode. The dots represent responses recorded 10 seconds apart.

Returning to the initial level of stimulation through electrode 1, the cell has a long-lasting increased sensitivity to stimulation.

E = T3

= c

o i

u u

> £

■"5 .5

Cfl -;

c c en ■-

Response to stimulating electrode 2



Q The sensitivity of the cell to stimulation through electrode 2 remains unchanged however.



Time

Conclusion: High frequency stimulation of synapses can cause a long-lasting change in sensitivity of these synapses—long-term potentiation (synaptic "memory").

44.20 Repeated Stimulation Can Cause Long-Term Potentiation

When a cell receives regular synaptic input, the resulting postsynaptic potential remains constant. If, however, that same synaptic pathway is stimulated briefly at a high frequency, the subsequent sensitivity of the postsynaptic cell to the original level of synaptic input is potentiated for a long time.

CjSL[^]tive transport by nearby cell memb ranes. The mode of action of JErozac, a commonly prescribed drug for depression, is to slow the reuptake of the neurotransmitter [^]exoton-in, thus enhancing its activity at the synapse.

Neurons in Networks

Because neurons can interact in the complex ways we have just discussed, network s of neurons can process and integrate information. Multiple neuronal networks constitute the nervous systems of animals. In subsequent chapters, we will see many examples of how neurons work together in networks to accomplish specific tasks. These networks use all of the mechanisms we have discussed: excitatory and inhibitory synapses, presyna ptic excitatio n and inhibition, and mechanisms of long-term po tent iation (and depression). Through these operations, our brains solve puzzles, create inventions, remember experiences, fall in love, and learn about biology. The challenge for the future is to understand how 7 these networks work.

Chapter Summary

Nervous Systems: Cells and Functions

- ▶ Nervous systems consist of cells that process and transmit information.
- ▶ Sensory cells transduce information from the environment and the body and communicate commands to effectors such as muscles or glands.
- ▶ The nervous systems of different species vary, but all are composed of cells called neurons. Review Figures 44.1, 44.2
- ▶ In vertebrates, the brain and spinal cord form the central nervous svstem, which communicates with other tissues of the body via the peripheral nervous system.
- ▶ Neurons receive information mostly via their dendrites and transmit information over their axons. Neurons function in networks. Review Figure 44.3
- ▶ The information that neurons process is in the form of electrical events in their plasma membranes. Where neurons and other cells meet, information is transmitted between them, mostly by the release of chemical signals called neurotransmitters.
- ▶ Glial cells physically support neurons and perform many housekeeping functions. Schwann cells and oligodendrocytes produce myelin, which insulates neurons. Astrocytes create the blood-brain barrier. Review Figure 44.4

Neurons: Generating and Conducting Nerve Impulses

- ▶ Neurons have an electric charge difference across their plasma membranes. This resting potential is created by ion pumps and ion channels. Review Figure 44.5

NEURONS AND NERVOUS SYSTEMS 793

- ▶ The sodium-potassium pump concentrates K⁺ on the inside of neurons and Na⁺ on the outsides. Ion channels allow K⁺ to leak out, leaving behind unbalanced negative charges and leading to the resting potential. Review Figures 44.6, 44.7

- ▶ A potassium equilibrium potential exists when the electric charge that develops across the membrane is sufficient to prevent a net diffusion of K^+ down its concentration gradient. This potential can be calculated with the Nernst equation. Review Figure 44.8
- ▶ The resting potential is perturbed when ion channels open or close, thus changing the permeability of the plasma membrane to charged ions. Through this mechanism, neurons become depolarized or hyperpolarized in response to stimuli. Review Figure 44.9
- ▶ Rapid reversals in charge across portions of the plasma membrane resulting from the opening and closing of voltage-gated sodium and potassium channels produce action potentials. These changes in voltage-gated channels occur when the plasma membrane depolarizes to a threshold level. Review Figure 44.10
- ▶ Action potentials are conducted down axons because of local current flow, which depolarizes adjacent regions of membrane and brings them to threshold for the opening of voltage-gated sodium channels. Review Figure 44.11
- ▶ Patch clamping allows us to study single ion channels. Review Figure 44.12
- ▶ In myelinated axons, the action potentials appear to jump between nodes of Ranvier, patches of plasma membrane that are not covered by myelin. Review Figure 44.13

Neurons, Synapses, and Communication

- ▶ Neurons communicate with each other and with other cells at specialized junctions called synapses, where the plasma membranes of two cells come close together.
- ▶ The classic chemical synapse is the neuromuscular junction, a synapse between a motor neuron and a muscle cell. Its neurotransmitter is acetylcholine, which causes a depolarization of the postsynaptic membrane when it binds to its receptor. Review Figure 44.14
- ▶ When an action potential reaches an axon terminal of the presynaptic cell, it causes the release of neurotransmitters, chemical signals that diffuse across the synaptic cleft and bind to receptors on the postsynaptic membrane. Review Figures 44.15, 44.16
- ▶ Synapses between neurons can be either excitatory or inhibitory. Synapses can also form on presynaptic membranes and thereby influence the release of neurotransmitter by the presynaptic cell.
- ▶ A postsynaptic neuron integrates information by summing its synaptic inputs in both space and time. Review Figure 44.17
- ▶ Ionotropic neurotransmitter receptors are ion channels. Metabotropic receptors influence the postsynaptic cell through various signal transduction pathways that involve G proteins. These pathways can result in changes in ion channels, alterations of enzyme activity, and even gene expression. The actions of ionotropic synapses are generally faster than those of metabotropic synapses. Review Figures 44.18, 44.19
- ▶ Electrical synapses pass electric signals between cells without the use of neurotransmitters.
- ▶ There are many different neurotransmitters and even more receptors. The action of a neurotransmitter depends on the receptor to which it binds. Review Table 44.1
- ▶ Glutamate binds to both ionotropic and metabotropic receptors, and may be involved in learning and memory. Review Figure 44.19
- ▶ With repeated stimulation, a neuron can become more sensitive to its inputs. Since this increased sensitivity can last a long time, it is called long-term potentiation, or LTP. The properties of the NMDA glutamate receptor appear to explain LTP. Review Figure 44.20
- ▶ In chemical synapses, the transmitter must be cleared rapidly from the synapse. Some poisons and some drugs act by blocking or slowing the clearance of transmitter from the synapse.

Neurons in Networks

- ▶ Neurons work together in networks to accomplish specific tasks. These networks use all of the mechanisms we have discussed in this chapter.

For Discussion

1. The language of the nervous system consists of one "word," the action potential. How can this single message convey a diversity of information, how can that information be quantitative, and how can it be integrated?
2. If you stimulate an axon in the middle, action potentials are conducted in both directions. Yet when an action potential is generated at the axon hillock, it goes only toward the axon terminals and does not backtrack. Explain why action potentials are bidirectional in the first example and unidirectional in the second.
3. The nature of synapses presents various opportunities for plasticity in the nervous system. Discuss at least four synaptic mechanisms that could be altered to change the response of a neuron to a specific input.

4. If Dr. Tsien had genetically engineered the AMPA receptor to remain open longer when activated, would it have made his mice smarter? Why or why not?

<S



Sensory Systems

How Animals perceive the world through their

Many senses. Different species look through different many sensory windows, so their views of the world are not the same. Dogs, for example, do not see color well, but they have keener senses of hearing and smell than humans do. While you enjoy a beautiful sunset, your dog might be enjoying sniffing around in the bushes and listening for the sounds of small animals in the underbrush.

Human hunters have exploited the remarkable sensory abilities of dogs for thousands of years. Most recently that hunt has extended to illicit drugs, smuggled contraband, bombs, and firearms. Dogs can be trained to detect the signature odors of such items, so they are used by police, customs agents, and other investigators to identify those odors wherever suspicious activities are occurring.

A black Labrador named Charlie (badge K9-001) was the first dog trained by the U.S. Treasury Department's Bureau of Alcohol, Tobacco, and Firearms to sniff out firearms and explosives. Charlie has sniffed out more than 200 illegal guns and 500 pounds of hidden explosives. With a nose that outperforms electronic sensors, Charlie helped solve a terrorist bombing case by discovering a tiny fragment of the bomb hundreds of yards from the site of the explosion. Charlie's nose is never off duty; on a recreational visit to a U.S. Civil War battlefield, it smelled out cannonball fragments that had been buried for 130 years. ATF dogs receive a lot of expert training, but their careers are based on their remarkable sense of smell.

In this chapter, we look at the general properties of sensory cells and see how they convert environmental stimuli to neural information. Sensory cells are generally called receptors, which creates some confusion with the receptor proteins that bind signaling molecules. To avoid this confusion, we use the terms sensory cells or receptor cells in this chapter. We examine in detail the cells responsible for chemoreception, mechanoreception, and photoreception and see how they are incorporated into sensory systems that provide the CNS with information about the world around and within us. In the course of our study of sensory systems, we will learn about the unusual sensory abilities of many animals.

Sensory Cells, Sensory Organs, and Transduction

Sensory cells transduce (convert) physical or chemical stimuli into signals that are transmitted to other parts of the nervous system for processing and interpretation. Most sensory cells are modified neurons, but some are other types of cells closely associated with neurons. Sensory cells are specialized for detecting specific kinds of stimuli, such as pressure, heat, or light.

Most sensory cells possess a membrane receptor protein that detects a stimulus and responds by altering the flow of ions across the plasma membrane (Figure 45.1). The resulting change in membrane potential causes the sensory cell either to fire action potentials itself or to change its secretory neurotransmitter onto an associated cell that fires ac-

tion potentials. The intensity of the stimulus is encoded in the frequency of action potentials.

Sensation depends on which neurons in the CNS receive action potentials from sensory cells

If the messages derived from all sensory cells are the same—action potentials—how can we perceive different sensations? Sensations such as heat, itch, pressure, pain, light, smell, and sound differ because the messages from sensory cells arrive at different places in the central nervous

;; .^v>w^

* . we**-

■ ■ * »-„•-.*.

jfrjtf

V

* t}

"?

■

Special Agent K9-001

Charlie's remarkable sense of smell enables him and his partner to discover illicit firearms and explosives.

Mechanoreceptor Pressure opens an ion channel.

Pressure

Thermoreceptor

Temperature influences a membrane enzyme that controls an ion channel.

Electroreceptor

An electric charge opens an ion channel.

Warmth

Chemoreceptor

A taste or smell molecule binds to a receptor, initiating a signal that controls the ion channel via intracellular messenger cascades.

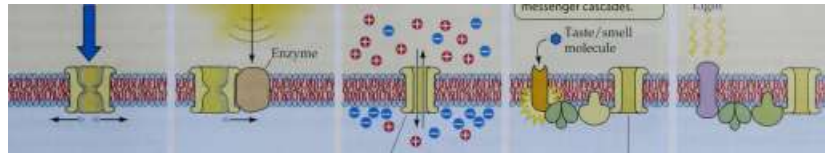
^ Taste/smell C molecule

Photoreceptor

Light alters the membrane protein, initiating a signal that controls an ion channel.

k

Light



Pressure-sensitive Na⁺ channel

Voltage-gated Na⁺ channel

Na⁺ or K⁺ channel

c GMP-mediated Na⁺ channel

45.1 Sensory Cell Membrane Receptor Proteins Respond to Stimuli

The receptors in mechanoreceptors, thermoreceptors, and electroreceptors are themselves ion channels. The activated receptor proteins of chemoreceptors and photoreceptors initiate biochemical cascades that eventually open or close ion channels.

system (CNS). Action potentials arriving in the visual cortex are interpreted as sight, in the auditory cortex as sound, in the olfactory bulb as smell, and so forth.

A small patch of skin on your arm contains sensory cells that increase their firing rates when the skin is warmed. Others increase their activity when the skin is cooled. Other types of sensory cells in the same patch of skin respond to touch, movement of hairs, irritants such as mosquito bites, and pain from cuts or burns. These sensory cells in your arm transmit their messages through axons that enter the CNS through the spinal cord. The synapses made by those axons in the spinal cord and the subsequent pathways of transmission determine whether the stimulation of the patch of skin on your arm is perceived as warmth, cold, pain, touch, itch, or tickle.

The specificity of these sensory circuits is dramatically illustrated in persons who have had a limb or part of a limb amputated. Although the sensory cells from that region are gone, the axons that communicated information from those sensory cells to the CNS may remain. If those axons are stimulated, the person feels specific sensations as if they were coming from the limb that is no longer there—a 'phantom limb'.

The messages from some sensory cells communicate information about internal conditions in the body, but we may not be consciously aware of that information. The brain receives continuous information about body temperature, blood sugar, blood carbon dioxide and oxygen concentration, arterial pressure, muscle tension, and the position of the limbs. All this information is important for the maintenance of homeostasis, but we don't have to think about it. If we did, there would be no time to think about anything else. All sensory cells produce information that

the nervous system can use, but that information does not always result in conscious sensation.

Sensory organs are specialized for detecting specific stimuli

Some sensory cells are assembled with other types of cells into sensory organs, such as eyes, ears, and noses, that enhance

the ability of the sensory cells to collect, filter, and amplify stimuli. A photoreceptor cell, for example, detects electromagnetic radiation of only a particular range of wavelengths. This selectivity is the basis for color vision and explains why some insects can see ultraviolet light. In some simple organisms photoreceptors sense only the presence of light, but in more complex animals, photoreceptors are combined with other cell types into eyes. We'll learn how eyes collect light and focus it onto sheets of photoreceptors so that patterns of light can be detected.

Sensory transduction involves changes in membrane potentials

In this chapter we examine several sensory cell types and the sensory organs with which they are associated. In each case we can ask the same general question: How does the sensory cell transduce energy from a stimulus into an action potential? The details differ for different sensory cells, but those details all fit into a general pattern.

We have already seen the first step of sensory transduction in Figure 45.1: the activation of a receptor protein. A receptor protein in the plasma membrane of the sensory cell is activated by a specific stimulus. The activated receptor protein opens or closes ion channels in the membrane by one of several mechanisms. The receptor protein may be part of an ion channel, much like an ionotropic synaptic receptor, and by changing its conformation it may open or close the channel directly. Alternatively, the activated receptor protein may function like a metabotropic synaptic receptor coupled to a G protein, setting off intracellular events that eventually affect ion channels (see Figures 15.8 and 15.10). Figure 45.2 reviews these first steps of sensory transduction and outlines the subsequent steps.

796 CHAPTER FORTY-FIVE

Stimulus

<t

| The stimulus activates a receptor protein by changing its conformation.

The activated receptor opens or closes ion channels.

A small stimulus can be amplified to produce a large response.

C3

Information about the stimulus is encoded as action potentials, or...

Qy...change in receptor potential causes the sensory cell to release neurotransmitter.

Sensory cell

Receptor protein

Ion channel

Receptor potential

Generator potential

Action potentials



Neurotransmitter release

4 .•.*:

^-.

9~»

Postsynaptic cell

generates action potentials

[Action potentials stimulate neurotransmitter release, thus transmitting information to other parts of the nervous system.

Neurotransmitter II Neurotransmitter release release

• •

&

45.2 Sensory Transduction Is a Series of Steps

The sensory cell itself may generate action potentials as a result of stimulation, or it may vary its release of neurotransmitter

in response to changes in its membrane potential, and the neurotransmitter may induce another cell to generate action potentials.

The opening or closing of ion channels in response to a stimulus changes the membrane potential of the sensory cell, which is called the receptor potential. Such changes in membrane potential can spread by current flow over short distances, but to travel long distances in the nervous system, receptor potentials must be converted into action potentials. The intracellular events involved in the conversion of the original stimulus-induced alteration of ion channels to action potentials can amplify the signal. In other words, the energy in the output of the sensory cell can be greater than the energy in the stimulus.

Receptor potentials produce action potentials in two ways: by generating action potentials within the sensory cell itself, or by releasing a neurotransmitter that induces an afferent synapse on a neuron, which generates action potentials (see Figure 45.2). In the first case, the sensory cell has a region of plasma membrane with voltage-gated sodium channels. A receptor potential that spreads to this region is called an action potential because it generates action potentials by causing the voltage-gated sodium channels to open.

A good example of generator potentials is found in the stretch receptors of crayfishes (Figure 45.3). By placing an electrode in the cell body of a crayfish stretch receptor cell, we can record the changes in the receptor potential that result from stretching the muscle to which the dendrites of the cell are attached. These changes in receptor potential become a generator potential at the base of the sensory cell's axon, where there are voltage-gated sodium channels. Action potentials generated here travel down the axon to the CNS. The rate at which action potentials are fired by the axon depends on the magnitude of the generator potential; that, in turn, depends on how much the muscle is stretched.

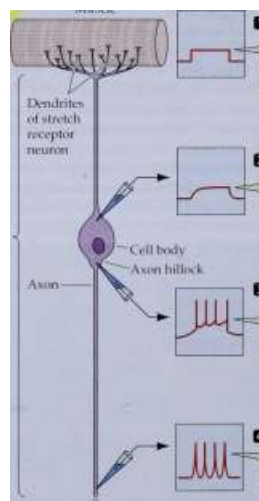
In sensory cells that do not fire action potentials, the spreading receptor potential reaches a presynaptic patch of plasma membrane and induces the release of a neurotransmitter.

Whether or not the sensory cell itself fires action potentials, ultimately the stimulus is transduced into action potentials and the intensity of the stimulus is encoded by the frequency of action potentials.

<&>'■

~~>

Muscle



- a.
- A
- en
- Dendrites of stretch receptor neuron
- Axon
- Stretching a muscle stimulates the opening of ion channels in stretch receptor dendrites.
- The resulting depolarization spreads to the cell body, creating a receptor potential...
- ...which becomes a generator potential at the axon hillock, causing an action potentials to fire.
- The action potential travels down the neuron.

45.3 Stimulating a Sensory Cell Produces a Generator Potential

The stretch receptor of a crayfish produces a generator potential when the muscle is stretched. At the axon hillock, the receptor produces action potentials

that travel down the axon.

SENSORY SYSTEMS 797

Many receptors adapt to repeated stimulation

Some sensory cells give gradually diminishing responses to maintained or repeated stimulation. This phenomenon is known as *sensory adaptation*, and it enables an animal to ignore background or unchanging conditions while remaining sensitive to changes or to new information. (Note that this use of the term "adaptation" is different from its application in an evolutionary context.) When you dress, you feel each item of clothing touch your skin, but the sensation of clothes touching your skin is not constantly on your mind throughout the day. You are immediately aware, however, when a seam rips, your shoe comes untied, or someone lightly touches your back.

The ability of animals to discriminate between continuous and changing stimuli is due partly to the fact that some sensory cells adapt; it is also due to information processing by the CNS. Some sensory cells adapt very little or very slowly; examples are pain receptors and mechanoreceptors for balance.[^]

In the rest of this chapter we will learn how sensory systems gather and filter stimuli, transduce specific stimuli into action potentials, and transmit action potentials to the CNS.

Chemoreceptors: Responding to Specific Molecules

Animals receive information about chemical stimuli through chemoreceptors. Chemoreceptors are responsible for smell, taste, and the monitoring of aspects of the internal environment such as the level of carbon dioxide in the blood. Chemosensitivity is universal among animals.

A colony of corals responds to a small amount of meat extract in the seawater around it by extending bodies and tentacles and searching for food. A solution of a single amino acid can stimulate this response. Humans have similar reactions to chemical stimuli. When we smell freshly

45.4 Some Scents Travel Great Distances

Mating in silkworm moths of the genus *Bombyx* is coordinated by a pheromone called bombykol.

baked bread, we salivate and feel hungry, but we gag and retch when we smell diamines from rotting meat. Information from chemoreceptors can cause powerful behavioral and physiological responses.

Arthropods provide good examples for studying chemosensation

Arthropods use chemical signals to attract mates. These signals, called pheromones, demonstrate the sensitivity of chemosensory systems. One of the best-studied examples of this phenomenon is the silk worm moth.

To attract a mate, the female silkworm moth releases a pheromone called bombykol from a gland at the tip of her abdomen. The male silkworm moth has receptors for this molecule on his antennae (Figure 45.4). Each feathery antenna carries about 10,000 bombykol-sensitive hairs. A single molecule of bombykol is sufficient to generate action potentials in the antennal nerve that transmits the signal to the CNS. Because of the male's high degree of sensitivity, the sexual message of a female moth is likely to reach any male that happens to be within a downwind area stretching over several kilometers. When approximately 200 hairs per second are activated, the male flies upwind in search of the female. Because the rate of firing in the male's sensory nerves is proportional to bombykol concentrations in the air, he can follow a concentration gradient and home in on the signaling female.

•4-

Many arthropods have chemoreceptor hairs, each containing one or more specific types of receptors. Crabs and flies, for example, have chemoreceptor hairs on their feet; these hairs have receptors for sugars, amino acids, salts, and other molecules. A fly tastes a potential food by stepping in it.

Olfaction is the sense of smell

The sense of smell, known as olfaction, also depends on chemoreceptors. In vertebrates, the olfactory sensors are neurons embedded in a layer of epithelial cells at the top of the nasal cavity. The axons of these neurons project to the olfactory bulb of the brain, and their dendrites end in olfac-

The female moth releases a pheromone from a gland at the tip of the abdomen.



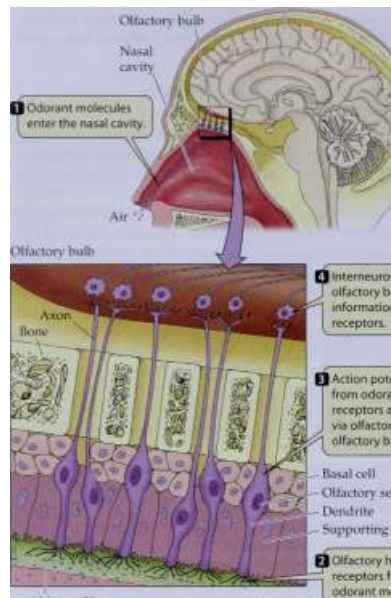
A male moth detects this pheromone in the air passing over his antennae, which are covered with chemosensitive hairs.



798 CHAPTER FORTY-FIVE

Brain

Olfactory



Interneurons in the olfactory bulb integrate information from olfactory receptors.

o Action potentials generated from odorant binding to the receptors are transmitted via olfactory sensors to the olfactory bulb.

Basal cell Olfactory sensor Dendrite Supporting cell

Mucus film

Olfactory hairs have rj receptors for specific odorant molecules.

45.5 Olfactory Receptors Communicate Directly with the Brain

The receptors of the human olfactory system are embedded in tissues lining the nasal cavity and send their axons to the olfactory bulb of the brain.

tory hairs at the surface of the nasal epithelium. A protective layer of mucus covers the epithelium. Molecules from the environment must diffuse through this mucus to reach the receptor proteins on the olfactory hairs. When you have a cold, the amount of mucus increases and the epithelium swells. With this in mind, study Figure 45.5 and you will easily understand why respiratory infections can cause you to lose your sense of smell.

A dog has up to 40 million nerve endings per square centimeter of nasal epithelium, many more than we do. Humans have a fairly sensitive olfactory system, but we are unusual among mammals in that we depend more on vision than on olfaction (we tend to join bird-watching societies more often than mammal-smelling societies). Whales and porpoises have no olfactory receptors and hence no sense of smell.

How does an olfactory sensory cell transduce the structure of a molecule from the environment into action poten-

tials? A molecule that triggers an olfactory receptor is called an o dorant. Odorants bind to receptor proteins on the olfactory hairs of the sensory cells. Olfactory receptor proteins are specific for particular odorant molecules—the two fit together like a lock and key.

If a "key" (an odorant molecule) fits the "lock" (the receptor protein), then a G protein is activated, which in turn activates an enzyme that causes an increase of a second messenger in the cytoplasm of the sensory cell. The second messenger binds to sodium channels in the sensory cell's plasma membrane and opens them, causing anmflux of NaVThe sensory cell thus

depolarizes to threshold and fires action potentials (see Figure 15.16).

The olfactory world has an enormous number of "keys"—molecules that produce distinct smells. The number of "locks"—receptor proteins—is large, but not nearly as large as the number of possible odorants. A family of about a thousand genes codes for olfactory receptor proteins. Each receptor protein is expressed in a limited number of sensory cells in the olfactory epithelium, and those cells all project to the same regions in the olfactory bulb. A given odorant molecule may bind to one or to more than one receptor protein. Therefore, each odorant molecule can excite a unique selection of cells in the olfactory bulb, so an olfactory system with a thousand different receptor proteins can discriminate a large number of smells.

How does the sensory cell signal the intensity of a smell? It responds in a graded fashion to the concentration of odorant molecules: The more odorant molecules that bind to receptors, the more action potentials are generated and the greater the intensity of the perceived smell.

Gustation is the sense of taste

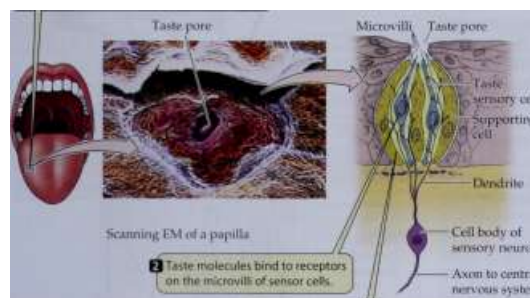
The sense of taste, or gustation, in humans and other vertebrates depends on clusters of sensory cells called taste buds. The taste buds of terrestrial vertebrates are confined to the mouth cavity, but some fishes have taste buds in the skin that enhance their ability to sense their environment. Some fishes living in murky water are very sensitive to small amounts of amino acids in the water around them and can find food without the use of vision. The duckbilled platypus, a monotreme mammal (see Figure 33.22b), has similar talents as a result of taste buds on the sensitive skin of its bill. What is a taste bud and how does it work?

A taste bud is a cluster of sensory cells (Figure 45.6). A human tongue has approximately 10,000 taste buds. The taste buds are embedded in the epithelium of the tongue, and many are found on the raised papillae of the tongue. (Look at your tongue in a mirror—the papillae make it look fuzzy.) Each papilla has many taste buds. The outer surface of a taste bud has a pore that exposes the tips of the sensory cells. Microvilli (tiny hairlike projections) increase the surface area of the sensory cells where their tips converge at the pore. These sensory cells, unlike olfactory receptors, are not

[I Taste bumps

buds are found on and around small (papillae) on the surface of the tongue.

SENSORY SYSTEMS 799



Microvilli Taste pore

Scanning EM of a papilla

?

Taste molecules bind to receptors on the microvilli of sensor cells.

Cell body of sensory neuron

Axon to central nervous system

Sensory cells use chemical signals to depolarize the dendrites of sensory neurons.

45.6 Taste Buds Are Clusters of Sensory Cells

Each taste bud contains a number of sensory cells that are not neurons.

neurons. At their bases, they form synapses with dendrites of sensory neurons.

Gustation begins with receptor proteins in the membranes of the microvilli. As with olfactory transduction, receptor proteins on the sensory cells bind specific molecules (such as sugar). This binding causes changes in the membrane potential of the sensory cells, which release neurotransmitters onto the dendrites of the sensory neurons. The sensory neurons fire action potentials that are conducted to the CNS.

The tongue does a lot of hard work, so its epithelium is shed and replaced at a rapid rate. Individual taste buds last only a few days before they are replaced, but the sensory neurons associated with them live on, always forming new synapses as new taste buds form.

You have probably heard that humans can perceive only four tastes: sweet, salty, sour, and bitter. In actuality, taste buds can distinguish among a variety of sweet-tasting molecules and a variety of bitter-tasting molecules. The full complexity of

the chemosensitivity that enables us to enjoy the subtle flavors of food comes from the combined activation of gustatory and olfactory receptors; that is the reason you lose some of your sense of taste when you have a cold.

Why does a snake continually sample the air by darting its forked tongue in and out? The forks of the snake's tongue fit into cavities in the roof of its mouth that are richly endowed with olfactory receptors. The tongue samples the air and presents the sample directly to the olfactory receptors. Thus the snake is really using its tongue to smell its environment, not to taste it. Why doesn't the snake simply use the flow of air to and from its lungs, as we do, to smell the environment? In reptiles, air flows to and from the lungs slowly (and can even stop entirely for long periods of time), but the tongue can dart in and out many times in a second. It is a quick source of olfactory information.

Mechanoreceptors: Detecting Stimuli that Distort Membranes

Mechanoreceptors are cells that are sensitive to mechanical forces. In the skin, different kinds of mechanoreceptors are responsible for the perception of touch, pressure, and tickle. Stretch receptors in muscles, tendons, and joints provide information about the position of the parts of the body in space and the forces acting on them. Stretch receptors in the walls of blood vessels signal changes in blood pressure. "Hair" cells with extensions that are sensitive to bending are incorporated into mechanisms for hearing and for signaling the body's position in space.

Physical distortion of a mechanoreceptor's plasma membrane causes ion channels to open, altering the membrane potential of the cell, which in turn leads to the generation of action potentials. The rate of action potentials tells the CNS the strength of the stimulus exciting the mechanoreceptor.

Many different sensory cells respond to touch and pressure

Objects touching the skin generate varied sensations because skin is packed with diverse mechanoreceptors (Figure 45.7). The outer layers of skin contain whorls of nerve endings enclosed in connective tissue capsules. These very sensitive mechanoreceptors, called Meissner's corpuscles, respond to objects that touch the skin even lightly. Meissner's corpuscles adapt rapidly. That is why you roll a small object between your fingers, rather than holding it still, to discern its shape and texture. As you roll it, you continue to stimulate these receptors anew.

Also in the outer regions of the skin are expanded-tip tactile receptors of various kinds. They differ from Meissner's corpuscles in that they adapt only partially and slowly. They are useful for providing steady-state information about objects that continue to touch the skin.

The density of these tactile mechanoreceptors varies across the surface of the body. A two-point discrimination

test demonstrates this fact.

If you lightly touch someone's back with two toothpicks, you can determine how far apart the two stimuli have to be before the person can distinguish whether he or she was touched with one or two points. On the back, the stimuli have to be rather far apart. The same test applied to the person's lips or fingertips reveals finer spatial discrimination; that is, the person can identify as separate two stimuli that are close together.

Deep in the skin, the dendrites of neurons wrap around hair follicles. When the hairs are displaced, those neurons are stimulated. Also deep within the skin is another type of mechanoreceptor, the Pacinian corpuscle. Pacinian corpuscles look like onions because they are made up of concentric layers of connective tissue, which encapsulate an extension of a sensory neuron. Pacinian corpuscles respond especially well to vibrations applied to the skin, but they adapt rapidly to steady pressure. The connective tissue capsule is important in this adaptation. An initial pressure distorts the corpuscle and the plasma membrane of the neuron at its core, but the layers of the capsule rapidly rearrange to redistribute the force, thus eliminating the distortion of the neuronal plasma membrane.

Meissner's corpuscle:

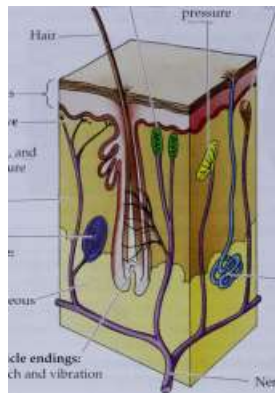
Light touch

Ruffini's corpuscle:

Touch and pressure

Krause's end bulb:

Touch



Epidermis

Bare nerve endings:

Pain, itch, and temperature

Dermis

Pacinian corpuscle:

Pressure

Subcutaneous tissue

Hair follicle endings

Light touch and vibration

Sweat gland

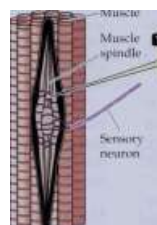
Nerve

45.7 The Skin Feels Many Sensations

Even a very small patch of skin contains a diversity of sensory cells.

(a) Muscle spindles

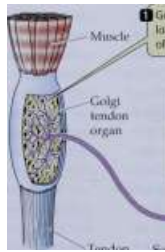
-Muscle



(b) Golgi tendon organs

Muscle spindles are stretch receptors. When muscle spindles stretchTTT

Time



Golgi tendon organs sense load and measure the force of muscle contraction.

Time

Tendon Sensory neuron

I.. sensory neurons associated with them transmit action potentials to the CNS. These signals stimulate motor neurons that initiate muscle contraction.

Q When contraction becomes too forceful...

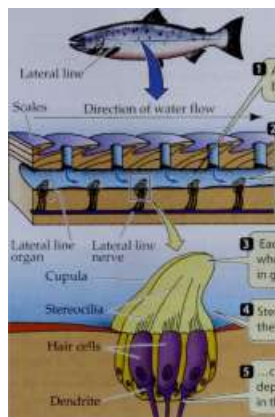


...the sensory neurons send action potentials to the CNS that inhibit the motor neurons, and the muscle relaxes.

45.8 Stretch Receptors Are Activated When Limbs Are Stretched

Stretch receptors provide information about the stresses on muscles and joints in an animal's limbs, (a) Signals from muscle spin-

dles to the CNS initiate muscle contraction. (fc>) Golgi tendon organs in tendons and ligaments inhibit a contraction that becomes too forceful, triggering relaxation and protecting the muscle from tearing.



A lateral line canal lies just below the skin surface.

Structures called cupulae project into the lateral line canal. As the fish moves through the water, fluid in the lateral line canal pushes against the cupulae.

Each cupula contains hair cells whose stereocilia are embedded in gelatinous material.

Stereocilia on hair cells in the cupula bend...

...creating a signal that causes depolarization of the dendrites in the lateral line nerve.

45.9 The Lateral Line System Contains Mechanoreceptors

Hair cells in the lateral line of a fish detect movement of the water around the animal, giving the fish information about its own movements and the movements of objects nearby.

Stretch receptors are found in muscles, tendons, and ligaments

An animal receives information from stretch receptors about the position of its limbs and the stresses on its muscles and joints. These mechanoreceptors are activated by being stretched. They continuously feed information to the CNS, and that information is essential for the coordination of movements.

The stretch receptors in skeletal muscle are called muscle spindles. They are embedded in connective tissue within skeletal muscle. They consist of modified muscle fibers that are innervated in the center with extensions of sensory neurons. Whenever the muscle stretches, muscle spindles are also stretched and the neurons transmit action potentials to the CNS (Figure 45.8a). Earlier in this chapter, we saw how crayfish stretch receptors transduce physical force into action potentials (see Figure 45.3). The actions of muscle spindles are similar.

Another stretch receptor, the Golgi tendon organ, is found in tendons and ligaments. It provides information about the force generated by a contracting muscle. When a contraction becomes too forceful, the information from the Golgi tendon organ feeds into the spinal cord, inhibits the motor neuron, and causes the contracting muscle to relax, thus protecting the muscle from tearing (Figure 45.8b).

Hair cells provide information about balance, orientation in space, and motion

Hair cells are also mechanoreceptors. Projecting from the surface of each hair cell is a set of stereocilia, which looks like a set of organ pipes. When these stereocilia (which are

SENSORY SYSTEMS 801

really microvilli) are bent, they alter receptor proteins in the hair cell's plasma membrane. When the stereocilia of some hair cells are bent in one direction, the receptor potential becomes more negative; when they are bent in the opposite direction, it becomes more positive. When the receptor potential becomes more positive, the hair cell releases neurotransmitter to the sensory neuron associated with it and the sensory neuron sends action potentials to the CNS.

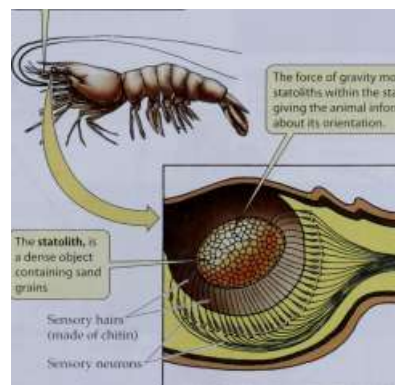
Hair cells are found in the lateral line sensory system of fish. The lateral line consists of a canal just under the surface of the skin that runs down each side of the fish (Figure 45.9). The lateral line provides information about movements of the fish through the water, as well as about moving objects, such as predators or prey, that cause pressure waves in the surrounding water.

Many invertebrates have equilibrium organs called statocysts that use hair cells to signal the position of the animal with respect to gravity (Figure 45.10). A statocyst is a chamber lined with hair cells that contains a dense object called a statolith. As the animal changes its position, the statolith moves in response to gravity, stimulating the hair cells below it. Replacing the statoliths of a lobster with iron filings and holding a magnet over the animal causes it to swim upside down. When a magnet is held to the lobster's side, it swims on its side.

Vertebrates also have equilibrium organs. The mammalian inner ear has two equilibrium organs that use hair cells to detect the position of the body with respect to gravity: semicircular canals and the vestibular apparatus. The

The statocyst is a chamber lined with hair cells located at the base of the antennule.

The force of gravity moves statoliths within the statocyst, giving the animal information about its orientation.



The statolith, is a dense object containing sand grains

Sensory hairs (made of chitin)

Sensory neurons

45.10 How a Lobster Knows Which Way Is Up

The statocyst is an equilibrium-sensing organ found in many invertebrates.

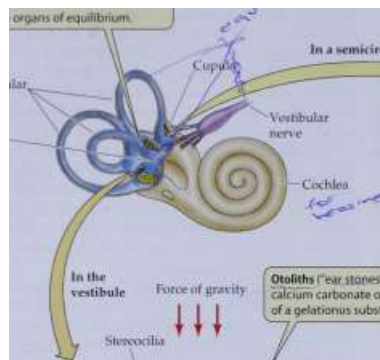
802 CHAPTER FORTY-FIVE

In the inner ear, the semicircular canals and the vestibule house the organs of equilibrium.

In a semicircular canal

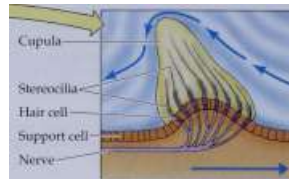
Semicircular, canals

Vestibule



In the semicircular canals, the gelatinous cupulae are pushed one way or the other when changes in the position of the head causes the fluid in the canals to shift.

Stereocilia-Hair cell-Support cell-Nerve-



Flow of fluid through semicircular canal

Stereocilia

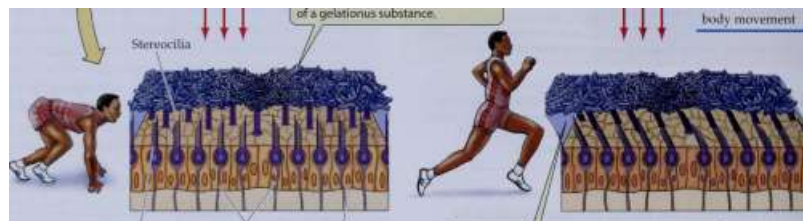
Force of gravity

Otoliths ("ear stones") are granules of calcium carbonate on the top surface of a gelatinous substance.

Direction of body movement

Force of gravity

Direction of body movement



Hair cell

Dendrites of sensory nerves

Support cell

45.11 Organs in the Inner Ear of Mammals Provide the Sense of Equilibrium

The bony inner ear has three parts: the snail-shaped cochlea, the semicircular canals, and the vestibule. The cochlea is for hearing; the semicircular canals and the vestibular apparatus provide the sense of equilibrium.

Layers of otoliths are moved by gravity when the head changes position, or they are moved by their inertia if the head accelerates or decelerates.

I Sound pressure waves travel through the auditory canal and vibrate the tympanic membrane.

A

\hat{r}

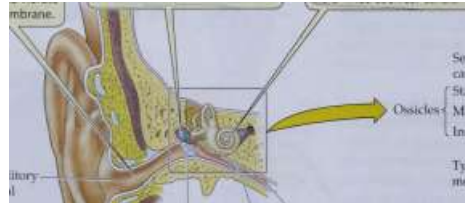
\hat{E}

Auditory canal

Pinna

The ossicles transmit vibrations of the tympanic membrane to the oval window of the cochlea...

Q.. where they are transduced into pressure waves in the fluid—filled cochlear canals.



c. \ r Tympanic Cochlea Eustachian

-V'

membrane

tube

EjThe pressure waves are transduced by mechanosensors into action potentials transmitted in the auditory nerve.

Semicircular canal C Stapes

Ossicles^ Malleus I Incus

Tympanic membrane



Oval window Round Cochlea

(attached to stapes) window

Outer ear

Middle Inner ear ear

rji^ 45.12 Structures of the Human Ear

The human ear uses hair cells to transduce sound waves into action potentials.



SENSORY SYSTEMS 803

inner ear contains three semicircular canals at right angles to one another. The structure and function of these organs are described in Figure 45.11. The vestibular apparatus has two chambers that perform a function similar to that of the statocysts of invertebrates.

Auditory systems use hair cells to sense sound waves

The stimuli that animals perceive as sounds are pressure waves. Auditory systems use mechanoreceptors to transduce pressure waves into action potentials. Auditory systems include special structures to gather sound waves, direct them to the sensory organ, and amplify their effect on the mechanoreceptors.

Human hearing provides a good example of an auditory system. The organs of hearing are the ears. The two prominent structures on the sides of our heads usually thought of as ears are the ear pinnae. The pinna of an ear collects sound waves and directs them into the auditory canal, which leads to the actual hearing apparatus in the middle ear and the inner ear (Figure 45.12). If you have ever watched a rabbit, a horse, or a dog change the orientation of its ear pinnae to focus on a particular sound, then you have witnessed the role of ear pinnae in hearing.

The eardrum, or tympanic membrane, covers the end of the auditory canal. The tympanic membrane vibrates in response to pressure waves traveling down the auditory canal. The middle ear, an air-filled cavity, lies on the other side of the tympanic membrane.

The middle ear is open to the throat at the back of the mouth through the eustachian tube. Because air flows through the eustachian tube, pressure equilibrates between the middle ear and the outside world. When you have a cold or allergy, the tube can become blocked by mucus or by tissue swelling, so you have difficulty "clearing your

ears," or equilibrating the pressure in the middle ear with the outside air pressure. As a result, the flexible tympanic

membrane bulges in or out, dampening your hearing and sometimes causing earaches.

The middle ear contains three delicate bones called the ear ossicles, individually named the malleus (hammer), incus (anvil) and stapes (stirrup). The ossicles transmit the vibrations of the tympanic membrane to another flexible membrane called the oval window. The leverlike action of the ossicles amplifies the vibrations of the tympanic membrane about 20-fold in transmitting them to the oval window membrane. Behind the oval window lies the fluid-filled inner ear. Movements of the oval window result in pressure changes in the inner ear. These pressure waves are transduced into action potentials.

The inner ear is a long, tapered, coiled chamber called the cochlea (from Latin and Greek words for "snail" or "shell").

A cross section of this chamber reveals that it is composed of three parallel canals separated by two membranes: Reissner's membrane and the basilar membrane (see Figure 45.12). Sitting on the basilar membrane is the organ of Corti, the apparatus that transduces pressure waves into action potentials in the auditory nerve, which in turn conveys information from the ear to the brain. The organ of Corti contains hair cells whose stereocilia are in contact with an overhanging, rigid shelf called the tectorial membrane. Whenever the basilar membrane flexes, the tectorial membrane bends the hair cell

stereocilia. As a consequence, the hair cells depolarize or hyperpolarize, altering the rate of action potentials transmitted to the brain by their associated sensory neurons.

Stereocilia of a hair cell



f

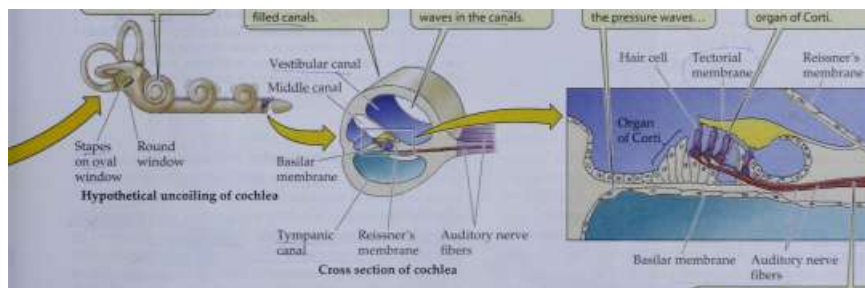
The cochlea is a tapered, coiled tube.

o ...that is divided length wise into three fluid filled canals.

Q Movements of the oval window create pressure waves in the canals.

fj The basilar membrane flexes in response to the pressure waves...

Q ...bending stereocilia on hair cells in the organ of Corti.



Hypothetical uncoiling of cochlea

Tympanic

canal

Reissner's membrane

Cross section of cochlea

(E) The movements of stereocilia are transduced into action potentials in the auditory nerve.

804 CHAPTER FORTY-FIVE

What causes the basilar membrane to flex, and how does this mechanism distinguish sounds of different frequencies? In Figure 45.13, the cochlea is shown uncoiled to make it easier to understand its structure and function. To simplify matters, we have left out Reissner's membrane, thus combining the upper and the middle canals into one upper canal. The purpose of Reissner's membrane is to contain a specific aqueous environment for the organ of Corti separate from the aqueous environment in the rest of the cochlea.

The simplified diagram of the cochlea shown in Figure 45.13 reveals two additional features that are important to its function. First, the upper and lower chambers separated by the basilar membrane are joined at the distal end of the cochlea (the end farthest from the oval window), making one continuous canal that folds back on itself. Second, just as the oval window is a flexible membrane at the beginning of the cochlea, the round window is a flexible membrane at the end of the long cochlea.

canal.

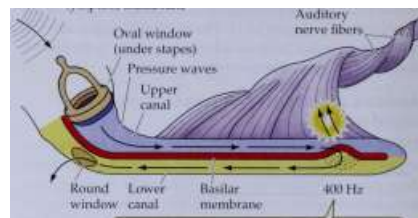
Air is highly compressible, but fluids are not. Therefore, a sound pressure wave can travel through air without much displacement of the air, but a sound pressure wave in fluid causes displacement of the fluid. When the stapes pushes the oval window in, the fluid in the upper canal of the cochlea is displaced. The cochlear fluid displacement travels down the upper canal, around the bend, and back through the lower canal. At the end of the lower canal, the displacement is absorbed by the outward bulging of the round window. ~~~~

If the oval window vibrates in and out rapidly, the waves of fluid displacement do not have enough time to travel all the way to the end of the upper canal and back through the lower canal. Instead, they take a shortcut by crossing the basilar membrane, causing it to flex. The more rapid the vibration, the closer to the oval and round windows the wave of displacement will flex the basilar membrane. Thus different pitches of sound flex the basilar membrane at different locations and activate different sets of hair cells (see Figure 45.13).

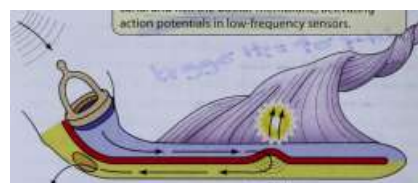
The ability of the basilar membrane to respond to vibrations of different frequencies is enhanced by its structure. Near the oval and round windows, at the proximal end, the basilar membrane is narrow and stiff, but it gradually becomes wider and more flexible toward the opposite (distal) end. So it is easier for the proximal basilar membrane to resonate with high frequencies and for the distal basilar membrane to resonate with lower frequencies. A complex sound made up of many frequencies distorts the basilar membrane at many places simultaneously and activates a unique subset of hair cells. Action potentials stimulated by the mechanoreceptors at different positions along the organ of Corti travel to the brain stem along the auditory nerve.

Deafness^ the loss of the sense of hearing, has two general causes. Conduction deafness is caused by the loss of function of the tympanic membrane and the ossicles of the middle ear. Repeated infections of the middle ear can cause scarring of the tympanic membrane and stiffening of the

Vibrations from the tympanic membrane



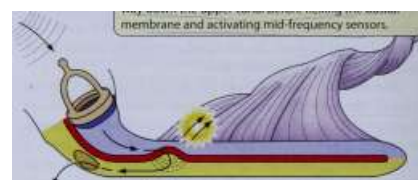
Low pitch: Pressure waves travel far down the upper canal and flex the basilar membrane", activating action potentials in low-frequency sensors.



3,000 Hz

J\

Medium pitch: Pressure waves travel only part of the way down the upper canal before flexing the basilar membrane and activating mid-frequency sensors.



■k

22,000 Hz

High pitch: Pressure waves travel a short distance before flexing the basilar membrane and activating high-frequency sensors.

45.13 Sensing Sound Pressure Waves in the Inner Ear

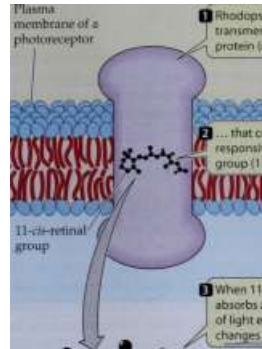
For simplicity, this diagram illustrates the cochlea as uncoiled, and leaves out Reissner's membrane. Pressure waves of different frequencies flex the basilar membrane at different locations. Information about sound frequency is specified by which hair cells are activated.

connections between the ossicles. The consequence is less efficient conduction of sound waves from the tympanic membrane to the oval window. With increasing age, the ossicles progressively stiffen, resulting in a gradual loss of the ability to hear high-frequency sounds. Nerve deafness is caused by damage to the inner ear or the auditory pathways. A common cause of nerve deafness is damage to the hair cells of the delicate organ of Corti by exposure to loud sounds such as jet engines, pneumatic drills, or highly amplified rock music. This damage is cumulative and permanent.

Plasma

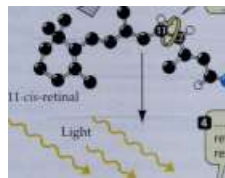
membrane of a photoreceptor

Rhodopsin is a transmembrane protein (opsin)...



... that contains a light-responsive prosthetic group (11-c/s-retinal).

m



When 11-c/s-retinal absorbs a photon of light energy, it changes shape...

^"w*** _

AN

v^--~



t...becoming all-trans-retinal, which is not responsive to light.

All-trans-retinal

45.74 Rhodopsin: A Photosensitive Molecule

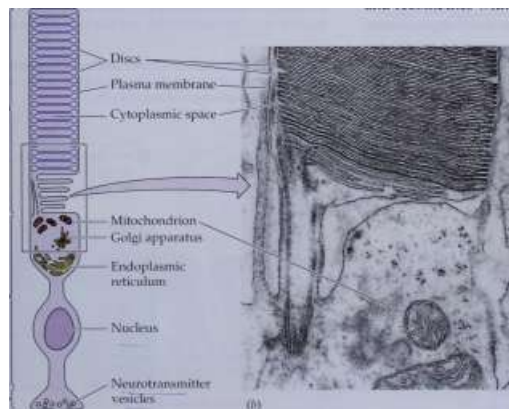
Rhodopsin changes its conformation when it absorbs light.

The molecule returns to the 11-c/s conformation and is photoresponsive again.

Outer segment

Inner segment

Synaptic (a) terminal



SENSORY SYSTEMS 805

Photoreceptors and Visual Systems: Responding to Light

Sensitivity to light—\photosensitivityj-coniers on the simplest animals the ability to orient to the sun and sky and gives more complex animals rapid and extremely detailed information about objects in their environment. It is not surprising that both simple and complex animals can sense and respond to light. What is remarkable is that across the entire range of animal species, evolution has conserved the same basis for photosensitivity: the family of pigments called rhodopsins.

In this section -we will learn how rhodopsin molecules respond when stimulated by light energy and how that response is transduced into neural signals. We will also examine the structures of eyes, the organs that gather and focus light energy onto photoreceptor cells.

Rhodopsin is responsible for photosensitivity

Photosensitivity depends on the ability of rhodopsins to absorb photons of light and to undergo a change in conformation. A rhodopsin molecule consists of a protein, opsin (which by itself does not absorb light), and a light-absorbing prosthetic group, 11-cis-retinal. The light-absorbing group is cradled in the center of the opsin and the entire rhodopsin molecule sits within the plasma membrane of a photoreceptor cell (Figure 45.14).

When the 11-cis-retinal absorbs a photon of light energy, its shape changes into a different isomer of retinal—all-trans-retinal. This change puts a strain on the bonds between retinal and opsin, changing the conformation of opsin. This change in conformation signals the detection of light. In vertebrate eyes, the retinal and the opsin eventually separate from each other—a process called bleaching, which causes the molecule to lose its photosensitivity. When the retinal spontaneously returns to its 11-cis isomer and recombines with opsin, it once again becomes photosensitive rhodopsin.

How does the light-induced conformational change of rhodopsin transduce light into a cellular response? After retinal converts from the 11-cis into the all-trans form, its interactions with opsin pass through several unstable intermediate stages. One of these stages is known as

45.75 The Rod Cell: A Vertebrate Photoreceptor

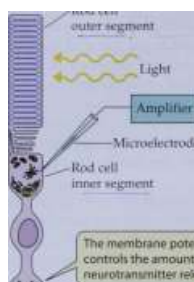
(a) The rod cell of the vertebrate retina is a neuron modified for photosensitivity. The membranes of a rod cell's discs are densely packed with rhodopsin. (b) A transmission electron micrograph of a section through a photoreceptor.

806 CHAPTER FORTY-FIVE

(a)

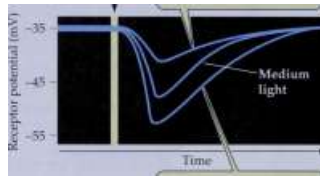
(b)

- Rod cell outer segment



Light flash

A dim light stimulus results in a slight hyperpolarization.



ie membrane potential controls the amount of neurotransmitter released.

A bright light stimulus results in a strong hyperpolarization.

45.76 A Rod Cell Responds to Light

The plasma membrane of a rod cell hyperpolarizes more negative—in response to a flash of light.

-becomes



^^

photoexcited rhodopsin because it triggers a cascade of reactions that results in the alteration of membrane potential that is the photoreceptor cell's response to light.

To get a better idea of how rhodopsin alters the membrane potential of a photoreceptor cell and how that photoreceptor cell signals that it has been stimulated by light, let's look at a vertebrate photoreceptor cell, the rod cell. Like other vertebrate photoreceptor cells, the rod cell is a modified neuron (Figure 45.15). The back of the vertebrate eye is the retina, which consists of several layers of __neuron __. One of these layers contains the photoreceptor cells. The other layers of the retina transduce the visual world into action potentials.

Each rod cell in the retina has an outer segment, an inner segment, and a synaptic terminal. The inner segment contains the usual organelles of a cell. The synaptic terminal is

where the rod cell communicates with other neurons. The outer segment is highly specialized and contains a stack of discs of plasma membrane densely packed with rhodopsin. The function of the discs is to capture photons of light passing through the rod cell.

To see how a rod cell responds to light, we can penetrate a single rod cell with an electrode and record its receptor potential in the dark and in the light (Figure 45.16a). From what we have learned about other types of sensory cells, we might expect stimulation of the rod cell by light to make its receptor potential less negative. But photoreceptor cells are atypical, and the opposite is true. When a rod cell is kept in the dark, it already has a relatively depolarized resting potential in comparison with other neurons. In fact, the plasma membrane of the rod cell is almost as permeable to Na^+ ions as to K^+ ions, and Na^+ ions are continually entering the outer segment of the cell.

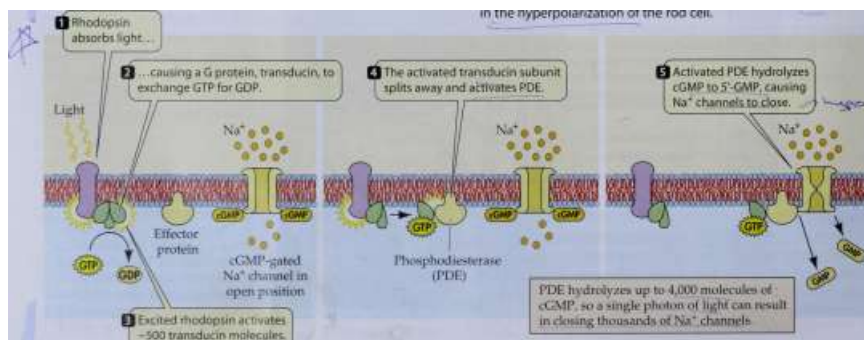
When a light is flashed on the dark-adapted rod cell, its receptor potential becomes more negative—it hyperpolarizes (Figure 45.16b). The rod cell itself does not generate action potentials. However, the rod cell changes its rate of neurotransmitter release as its membrane potential changes (since the rod cell hyperpolarizes, neurotransmitter released decreases). Later in this section we will learn how other cells in the retina respond to neurotransmitter released from the photoreceptor cells.

How does the absorption of light by rhodopsin hyperpolarize the rod cell? When rhodopsin is excited by light, it initiates a cascade of events. The photoexcited rhodopsin combines with and activates another protein, a G protein

45.17 Light Absorption Closes Sodium Channels

The absorption of light by rhodopsin initiates a cascade resulting in the hyperpolarization of the rod cell.

Activated PDE hydrolyzes cGMP to 5'-GMP, causing Na^+ channels to close. ...



^-^^T'C^SL-VZ.^

PDE hydrolyzes up to 4,000 molecules of cGMP, so a single photon of light can result in closing thousands of Na^+ channels.

L

Flatworm responds to light by moving directly away from the source toward darkness.



Light-sensitive regions containing rhodopsin



45.18 A Simple Photosensory System

Although flatworms do not "see" as we do, their eye cups enable them to move away from a light source to an area where they may be less visible to predators.

Nerves to brain

Pigmented eye cups

called transducin. Activated transducin in turn activates a phosphodiesterase, which converts cyclic GMP (cGMP) to 5'-GMP. This reaction plays a central role in phototransduction. In the dark, the cGMP in the outer segment binds to sodium channels, keeping them open and allowing Na^+ to enter the outer segment. As cGMP is converted to 5'-GMP, the sodium channels close, and the cell hyperpolarizes.

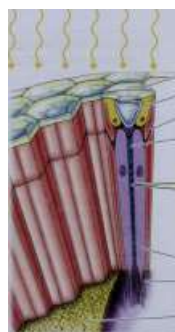
This may seem like a roundabout way of doing business, but its advantage is its enormous amplification ability. Each molecule of photoexcited rhodopsin can activate as many as 500 transducin molecules, thus activating a large number of phosphodiesterase molecules. The catalytic capacity of a molecule of phosphodiesterase is great: it can hydrolyze more than 4,000 molecules of cGMP per second. The bottom line is that a single photon of light can cause more than a million sodium channels to close, thereby changing the rod cell's receptor potential (Figure 45.17).

Now let's see how photoreceptors of different types are incorporated into different kinds of visual systems.

Invertebrates have a variety of visual systems

Flatworms obtain directional information about light from photoreceptor cells that are organized into eye cups (Figure

The compound eyes of a fruit fly each contain hundreds of ommatidia.



45.18). The eye cups are bilateral structures, each partly shielded from light by a layer of pigmented cells lining the cup. The photoreceptors on the two sides of the animal are unequally stimulated unless the animal is facing directly toward or away from a light source. The flatworm generally uses directional information about light sources to move away from light.

Arthropods (crustaceans, spiders, and insects) have evolved compound eyes that provide them with information about patterns or images in the environment. Each compound eye consists of many optical units called ommatidia (singular ommatidium) (Figure 45.19). The number of ommatidia in a compound eye varies from only a few in some ants, to 800 in fruit flies, to 10,000 in some dragon-flies.

Each ommatidium has a lens structure that directs light onto photoreceptors called retinal cells. Flies, for example, have seven elongated retinula cells in each ommatidium. The inner borders of the retinula cells are covered with microvilli that contain rhodopsin and thus trap light. Since the microvilli of the different retinula cells overlap, they appear to form a central

rod, called a rhabdom, down the center of the ommatidium.

Axons from the retinula cells communicate with the nervous system. Since each ommatidium of a compound eye is directed at a slightly different part of the visual world, only a crude, or perhaps a broken-up, image can be communicated from the compound eye to the CNS.

Image-forming eyes evolved independently in vertebrates and cephalopods

Both vertebrates and cephalopod mollusks have evolved eyes with exceptional abilities to form images of the visual world. Like cameras, these eyes focus images on a surface sensitive to light. Considering that they evolved independently of each other, their high degree of similarity is remarkable (Figure 45.20).

Corneal lens Crystalline cone Pigment cell Rhabdom

Each ommatidium focuses light on a rhabdom consisting of the overlapping, light-sensitive plasma membranes of a few retinula cells.

Retinula cell

Bundle of axons to brain

Basement membrane

(«)

(b)

45.19 Ommatidia: The Functional Units of Insect Eyes

(a) The micrograph shows the compound eye of a fruit fly (*Drosophila*). [b] The rhodopsin-containing retinula cells are the photoreceptors in ommatidia.



808 CHAPTER FORTY-FIVE

45.20 Eyes Like Cameras

The lenses of cephalopod and vertebrate eyes focus images on layers of photoreceptor cells, just as a camera's lens focuses images on film.

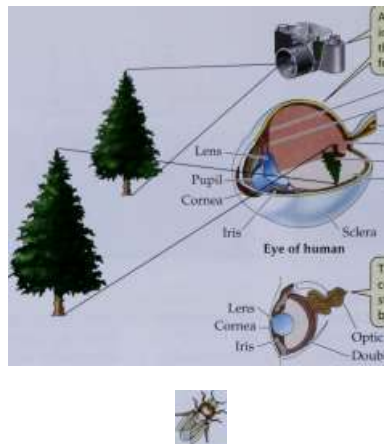
The vertebrate eye is a spherical, fluid-filled structure bounded by a tough connective tissue layer called the sclera. At the front of the eye, the sclera forms the transparent cornea through which light passes to enter the eye. Just inside the cornea is the pigmented iris, which gives the eye its color. The function of the iris is to control the amount of light that reaches the photoreceptor cells at the back of the eye, just as the diaphragm of a camera controls the amount of light reaching the film. The central opening of the iris is the pupil. The iris is under neural control. In bright light the iris constricts and the pupil is very small. As light levels fall, the iris relaxes and the pupil enlarges.

Behind the iris is the crystalline protein lens, which helps focus images on the photoreceptors at the back of the eye. The cornea and the fluids of the eye chambers are mostly responsible for focusing light on the retina, but the lens allows the eye to accommodate—that is, to focus on objects at various locations in the near visual field. To focus a camera on objects close at hand, you adjust the distance between the lens and the film. Fishes, amphibians, and reptiles accommodate in a similar manner, moving the lenses of their eyes closer to or farther from their retinas. Mammals and birds use a different method: they alter the shape of the lenses.

The lens is contained in a connective tissue sheath that tends to keep it in a spherical shape, but it is attached to suspensory ligaments that pull it into a flatter shape. Circular muscles called the ciliary muscles counteract the pull of the suspensory ligaments and permit the lens to round up. With the ciliary muscles at rest, the flatter lens has the correct optical properties to focus distant objects on the retina, but not close images. Contracting the ciliary muscles rounds up the lens, changing its light-bending properties to bring close images into focus (Figure 45.21). As we age, our lenses become less elastic and we lose the ability to focus on objects close at hand without the help of corrective lenses. As a consequence, most adults over the age of 45 need the assistance of bifocal lenses or reading glasses to compensate for their lost ability to accommodate.

The vertebrate retina receives and processes visual information

During development, neural tissue grows out from the brain to form the retina. In addition to a layer of photoreceptor cells, the retina includes layers of cells that process the visual information



A camera's lens focuses an inverted image on the film in the same way the eye's lens focuses an image on the retina.

Circular ciliary muscle

Suspensory ligaments

Optic nerve

Blind spot

Fovea

Retina

The eye of the squid, a cephalopod, is very similar in structure to the vertebrate eye, but it evolved independently.

Optic nerve

Double layer of receptor cells

Eye of squid

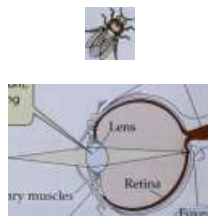
from the photoreceptors and produce an output signal that is transmitted to the brain via the optic nerve. A curious feature of the anatomy of the retina is that the light-absorbing outer segments of the photoreceptor cells are all the way at the back of the retina. Light must pass through all the layers of retinal cells before reaching the place where photons are captured by rhodopsin. We will explore in detail how the cells of the retina process information, but first let's describe some general features of retinal organization.

THE CELLULAR STRUCTURE OF THE RETINA. The density of

photoreceptor cells is not the same across the entire retina. Light coming from the center of the visual field falls on an area of the retina called the fovea, where the density of photoreceptor cells is the highest. The human fovea has about 160,000 photoreceptors per square millimeter. A hawk has about 1 million photoreceptors per square millimeter of fovea, making its vision sharper than ours. In addition, the hawk has two foveas in each eye: one receives light from

For near vision (accommodation), ciliary muscles contract, causing the lens to round up.

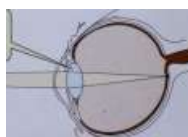
Optic nerve



Ciliary muscles / » Suspensory filaments

Fovea

For distant vision, ciliary muscles relax and suspensory ligaments pull the lens to a flatter shape.

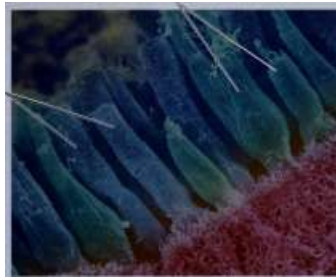


45.21 Staying in Focus

Mammals and birds focus their eyes by changing the shape of the lens.

Cone cells

Rod cells



45.22 Rods and Cones

This scanning electron micrograph of photoreceptors in the retina of a mud puppy (an amphibian) shows cylindrical rods and tapered cones.

straight ahead, while the other receives light from below. Thus while the hawk is flying, it sees both its projected flight path and the ground below, where it might detect a mouse scurrying in the grass.

The fovea of a horse is a long, vertical patch of retina. The horse's lens is not good at accommodation, but it focuses distant objects that are straight ahead on one part of this long fovea and close objects that are below the head on another part. When horses are startled by an object close at hand, they pull their heads back and rear up to bring the object into focus on the close-vision part of the fovea.

Where blood vessels and the bundle of axons going to the brain (the optic nerve) pass through the back of the eye, there are no photoreceptors, so there is a blind spot on the retina. You are normally not aware of your blind spot, but you can find it. Stare straight ahead, holding a pencil in your outstretched hand so that the eraser is in the center of your field of vision. While continuing to stare straight ahead, slowly move the pencil to the side until the eraser disappears. When this happens, the light from the eraser is focused directly on your blind spot.

Until now we have referred to only one kind of photoreceptor cell, the rod cell. But there are two major kinds of vertebrate photoreceptors, both named for their shapes—rod cells and cone cells (Figure 45.22). A human retina has about 3 million cones and about 100 million rods. Rod cells are more sensitive to light, but do not contribute to color vision! Some cone cells are responsible for color vision, but are less sensitive to light. Cones are also responsible for our sharpest vision. Even though there are many more rods than cones in human retinas, our foveas contain only cones.

Because cones have low sensitivity to light

they are of

no use in dim light. At night our vision is not very sharp and we see mostly in shades of gray. You may have trouble seeing a small object such as a keyhole at night when you are looking straight at it—that is, when its image is falling on your fovea. If you look a little to the side, so that the image falls on a rod-rich area of your retina, you can see the

SENSORY SYSTEMS 809

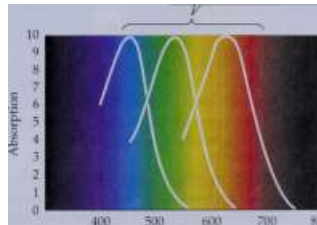
object better. Astronomers looking for faint objects in the sky learned this trick a long time ago. Animals that are nocturnal (such as mice or flying squirrels) may have retinas made up almost entirely of rods and have little or no color vision. By contrast, some animals that are active only during the day (such as chipmunks and ground squirrels) have only cones in their retinas.

How do cone cells enable us to see color? The human retina has three kinds of cone cells, each containing slightly different types of opsin molecules. These opsin molecules differ in the wavelengths of light they absorb best. Although the same 11-trans-retinal group is the light absorber (see Figure 45.14), its molecular interactions with opsin tune the spectral sensitivity of the rhodopsin molecule as a whole. Some opsins cause retinal to absorb most efficiently in the blue region, some in the green, and some in the yellow and red (Figure 45.23). Intermediate wavelengths of light excite these different classes of cones in different proportions. The genes that encode the different opsins of humans have been cloned: One codes for blue-sensitive opsin, one for green-sensitive opsin, and several for red-sensitive opsin.

The human retina is organized into five layers of neurons that receive visual information and process it before sending it to the brain (Figure 45.24). As mentioned earlier, the layer of photoreceptors is the way at the back of the retina. The outer segments of the rods and cones are partly buried in a layer of pigmented epithelium that absorbs photons not captured by rhodopsin and prevents any backscattering of light that might decrease visual sharpness.

INFORMATION FLOW IN the RETINA. A first step in investigating how the human retina tells the brain what it sees is to study how its five layers of neurons are interconnected and how they influence one another. As we know, the photoreceptor cells at the back of the retina hyperpolarize in response to light and do not generate action poten-

Human color vision is based on three kinds of cone cells. Each absorbs a different band of wavelengths most effectively.



500 600 700 800

Wavelength (nm)

45.23 Absorption Spectra of Cone Cells

The three kinds of cone cells contain slightly different opsin molecules, which absorb different wavelengths of light.

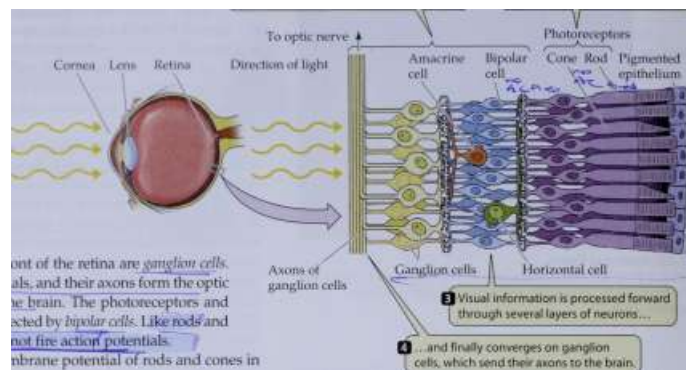
810 CHAPTER FORTY-FIVE

Q Light travels through layers of transparent neurons—ganglion, amacrine, bipolar, and horizontal cells...

o ... and is absorbed by discs in the rods and cones (the photoreceptive layer) at the back of the retina.

c omea Lens Rot inn

Cone Rod Pigmented N C** \ epithelium



M

tials. The cells at the front of the retina are gang lion cells. They fi re action potentials, and their axons form the optic nerves that travel to the b rain. The photoreceptors and ganglion cells are connected by bi polar cel ls. Like rods and cones, bipolar ce lls do not fire actioy! potent ials.

Changes in the membrane potential of rods and cones in response to light alter the rate at which the rods and cones release neuro transmit ter at their synapses with the bipolar cells. In response to neurotransmitter from the photoreceptors, the membrane potentials of the bi polar c eLte change, a ltering the rate at which they release neurotransm itter on to ganglion ce lls. The gang lion cells gene rate action pote ntials, and the rate of neurotransmitter releas e from the bipola r cells de t ermines the rate at w hich they do so. Thus the direct flow of information in the retina is from photo rece ptor to bipolar cell to ganglion cell. Ganglion cells send the information to the brain.

The other two cell layers, the horizontal cells and the amac rine c ells, communicate laterally across the retina. Hori-zontafcells connect neighboring pairs of photoreceptors and bipolar cells. Thus the communication between a photoreceptor and its bipolar cell can be influenced by the amount of li ght absorbed by neighboring photoreceptors. This lateral flow of information enables the retina to sharp en the perception of contrast between light and dark patterns.

(Amacrine ce Us\ connect neighboring pairs of bipolar cells and ganglion cells. The role of amacrine cells is still poorly understood. Some amacrine cell types are highly sensi tive to changing illumination or to motion. Others assist in adjusting the sensitivity of the eyes according to the overall level of light falling on the retina. When background light levels change, amacrine cell connections to the ganglion cells help the ganglion cells remain sensitive to temporal changes in stimulation. Thus even with large changes in background illumination, the eyes are sensitive to smaller, more rapid changes in the pattern of light falling on the retina.

Information processing in the retina. Knowing the paths of information flow through the retina still doesn't tell us how that information is processed. What does the eye tell the brain in response to a pattern of light falling on the ret ina? One aspect of info rmation processing in the retina \ \$folroergence of information }^ here are more than 100 mil-ljonpho toreceptors in each reti na, but only about 1 million

Axons of ganglion cells

t

Visual information is processed forward through several layers of neurons...

...and finally converges on ganglion cells, which send their axons to the brain.



t 45.24 The Retina

The human retina has five layers of neurons that receive and process visual information.

ganglion cells sending messages to the brain. How is the information from all those photoreceptors integrated by the ganglion cells?

This question was addressed in some elegant, classic experiments in which electrodes were used to record the activity of single ganglion cells in living animals while their retinas were stimulated with spots of light. These studies revealed that each ganglion cell has a well-defined receptive field that consists of a specific group of photoreceptor cells. Stimulating these photoreceptors with light activates the ganglion cell (Figure 45.25). Information from many photoreceptor cells is integrated in this way to produce a single message.

The receptive fields of many ganglion cells are circular, but the way a spot of light influences the activity of the ganglion cell depends on where in the receptive field it falls. The receptive field of a ganglion cell can be divided into two concentric areas, called the center and the surround. There are two kinds of receptive fields, on-center and off-center. Stimulating the center of an on-center receptive field excites the ganglion cell, and stimulating the surround inhibits it. Stimulating the center of an off-center receptive field inhibits the ganglion cell, and stimulating the surround excites it. Center effects are always stronger than surround effects.

The response of a ganglion cell to stimulation of the center of its receptive field depends on how much of the surround area is also stimulated. A small dot of light hitting the center has the maximal effect. A bar of light hitting the center and pieces of the surround has less of an effect, and a large, uniform patch of light falling equally on center and surround has very little effect. Ganglion cells commu-

nicate information about contrasts between light and dark that fall on different regions of their receptive fields.

How are receptive fields related to the connections among the neurons of the retina? The photoreceptors in the center of the receptive field of a ganglion cell are connected to that ganglion cell by bipolar cells. The photoreceptors in

^ 45.25 What Does the Eye Tell the Brain?

When the retina is stimulated with dots and rings of light, individual ganglion cells show different responses.



the surround send information to the center photoreceptors, and thus to the ganglion cell, through the lateral connections of horizontal cells. Thus the receptive field of a ganglion cell consists of a pattern of synapses among photoreceptors, horizontal cells, bipolar cells, and ganglion cells.

The receptive fields of neighboring ganglion cells can overlap greatly; a given photoreceptor can be effectively connected to several ganglion cells. Thus the ganglion cells send simple messages to the brain about the pattern of light

H

EXPERIMENT

Question: How do retinal ganglion cells code patterns of light falling on the retina?

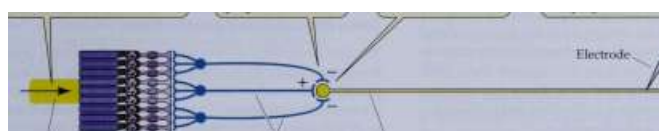
METHOD

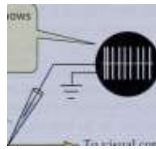
| Light stimulates a small, circular area of the retina. (In reality, the light would come from the other direction.)

I Photoreceptors in a circular receptive field give input to a single ganglion cell.

A retinal ganglion cell receives input from all photoreceptors in its receptive field.

EJ An oscilloscope shows action potentials generated by the ganglion cell.





t> To visual cortex

Pattern of light stimulating retina

RESULTS

Bipolar cells

Axon in optic nerve

Stimulus patterns

Complete darkness

Small spot falling on center of receptive field

Large spot covering receptive field

Ring of light excluding center of receptive field



On

StimulusN



An on-center ganglion cell is inhibited by a ring of light falling on its receptive field's surround.

An off-center ganglion cell is stimulated by light falling on its receptive field's surround and is inhibited by light falling on its center.

Conclusions: 1. Ganglion cells have circular receptive fields that are divided into center and surround areas.

2. Some ganglion cells are maximally stimulated by light falling on the center of their receptive fields. Others are maximally stimulated by light falling on the surround of their receptive fields.

3. Ganglion cells encode patterns of light and dark contrast.

812 CHAPTER FORTY-FIVE

Pit organ

intensities falling on small, circular patches of retina. In Chapter 47 we will see how the brain reassembles that information into our view of the world.

Sensory Worlds Beyond Our Experience

Humans make use of only a subset of the information available to us in the environment. Other animals have sensory systems that enable them to use different subsets and different types of information.

Some species can see infrared and ultraviolet light

When discussing vision, we use the term "visible light," but what we really mean is light visible to humans. Our visible spectrum is a very narrow region of the entire, continuous range of electromagnetic radiation in the environment (see Figure 8.5). We cannot see ultraviolet radiation, for example, but many other animals can.

One of the seven photoreceptors in each ommatidium of a fruit fly is sensitive to ultraviolet light. The visual sensitivity of many pollinating insects includes the ultraviolet part of the spectrum. Some flowers have patterns that are invisible to us but show up if we photograph them with film that is sensitive to ultraviolet light. Those patterns provide information to

prospective pollinators, but humans are not equipped to receive that information.

At the other end of the spectrum is infrared radiation, which we sense as heat. Other animals extract much more information from infrared radiation—especially that emitted by potential prey. Pit vipers such as rattlesnakes have pit organs, one just in front of each eye, that use highly sensitive heat detectors and a simple pinhole camera arrangement to sense and locate infrared radiation (Figure 45.26). In total darkness, these snakes can locate a prey animal such as a mouse, orient to it, and strike it with great accuracy.

Echolocation is sensing the world through reflected sound

Some species emit intense sounds and create images of their environments from the echoes of those sounds. Bats, porpoises, dolphins, and (to a lesser extent) whales are accomplished echolocators. Some species of bats have elaborate modifications of their noses to direct the sounds they emit, as well as impressive ear pinnae to collect the returning echoes. The high-frequency sounds they emit as pulses (about 20 to 80 per second) are above the range of human hearing, but they are extremely loud in contrast to the resulting faint echoes bouncing off small insects. An echolocating bat is similar to a construction worker who is trying to overhear a whispered conversation on a street corner while using a pneumatic drill. To avoid deafening themselves, bats use muscles in their middle ears to dampen their sensitivity while they emit sounds, then relax them quickly enough to hear the echoes. The ability of bats to use echolocation to sense their environment is so good that in a totally dark room strung with fine wires, they can capture tiny flying insects while navigating around the wires.



Crotalus molossus

45.26 Stalking in the Dark

The black-tailed rattlesnake of the southwestern United States is a pit viper. Pit vipers can locate prey in total darkness on the basis of infrared radiation they sense through their pit organs.

Some fish can sense electric fields

We discussed the mechanoreceptors in the lateral lines of fishes (see Figure 45.9). The lateral lines of some species, especially those such as catfish that live in murky waters, also contain electroreceptors. These sensory cells enable the fish to detect weak electric fields, which can help them locate prey.

The use of electroreceptors is even more sophisticated in species called electric fishes. These fishes have evolved electric organs in their tails that generate a continuous series of electric pulses, creating a weak electric field around their bodies. Any objects in the environment, such as rocks, plants, or other fish, disrupt the electric fish's electric field, and the electroreceptors of the lateral line detect those disruptions. In some electric fish species, each individual in a group emits its electric pulses at a different frequency. If a new fish is added to the group, they all readjust their frequencies.

Chapter Summary

Sensory Cells, Sensory Organs, and Transduction

- Sensory cells transduce information about an animal's external and internal environment into action potentials. Review Figures 45.1, 45.2
- The interpretation of action potentials as particular sensations depends on which neurons in the CNS receive them.
- Sensory cells have membrane receptor proteins that cause ion channels to open or close, generating receptor potentials. Receptor potentials can spread to regions of the sensory cell plasma membrane that generate action potentials, or they can influence the release of neurotransmitter from the sensory cell. Review Figure 45.3
- Adaptation enables the nervous system to ignore irrelevant stimuli while remaining responsive to relevant or to new stimuli.

Chemoreceptors: Responding to Specific Molecules

- Smell, taste, and the sensing of pheromones are examples of chemosensation. Chemoreceptor cells have receptor proteins that can bind to specific molecules that come into contact with the sensory cell membrane. Review Figure 45.5

SENSORY SYSTEMS 813

teins that can bind to specific molecules that come into contact with the sensory cell membrane. Review Figure 45.5

- The binding of an odorant molecule to a receptor protein causes the production of a second messenger in the chemoreceptor cell. The second messenger alters ion channels and creates a receptor potential. Review Figure 15.17

- Chemoreceptors in the mouth cavities of vertebrates are responsible for the sense of taste. Review Figure 45.6

Mechanoreceptors: Detecting Stimuli that Distort Membranes

- In the skin there are a diversity of mechanoreceptors that respond to touch and pressure. The density of mechanoreceptors in any skin area determines the sensitivity of that area to touch and pressure. Review Figure 45.7
- Stretch receptors in muscles, tendons, and ligaments inform the CNS of the positions of and the loads on parts of the body. Review Figure 45.8
- Hair cells are mechanoreceptors that are not neurons. The bending of their stereocilia alters their membrane proteins and therefore their receptor potentials. Hair cells are found in organs of equilibrium and orientation such as the lateral line system of fishes, the statocysts of invertebrates, and the semicircular canals and vestibular apparatus of mammals. Review Figures 45.9, 45.10, 45.11
- Hair cells are responsible for mammalian auditory sensitivity. Ear pinnae collect and direct sound waves to the tympanic membrane, which vibrates in response to sound waves. The movements of the tympanic membrane are amplified through a chain of ossicles that conduct the vibrations to the oval window. Movements of the oval window create pressure waves in the fluid-filled cochlea. Review Figure 45.12
- The basilar membrane running down the center of the cochlea is distorted at specific locations that depend on the frequency of the pressure wave. These distortions cause the bending of hair cells in the organ of Corti, which rests on the basilar membrane. Changes in hair cell receptor potentials create action potentials in the auditory nerve, which conducts the information to the CNS. Review Figure 45.13

Photoreceptors and Visual Systems: Responding to Light

- Photosensitivity depends on the capture of photons of light by rhodopsin, a photoreceptor molecule that consists of a protein called opsin and a light-absorbing prosthetic group called retinal. Absorption of light by retinal is the first step in a cascade of intracellular events leading to a change in the receptor potential of the photoreceptor cell. Review Figure 45.14
- When excited by light, vertebrate photoreceptor cells hyperpolarize and release less neurotransmitter onto the neurons with which they form synapses. They do not fire action potentials. Review Figures 45.15, 45.16, 45.17
- Vision results when eyes focus patterns of light onto layers of photoreceptors. Eyes vary from the simple eye cups of flatworms, which enable the animal to sense the direction of a light source, to the compound eyes of arthropods, which enable the animal to detect shapes and patterns, to the lensed eyes of cephalopods and vertebrates. Review Figures 45.18, 45.19
- The eyes of vertebrates and cephalopods focus detailed images of the visual field onto dense arrays of photoreceptors that transduce the visual image into neural signals. Review Figures 45.20, 45.21
- The vertebrate photoreceptors are rod cells, responsible for dim light and black-and-white vision, and cone cells, responsible for color vision by virtue of their spectral sensitivities. Review Figure 45.23
- The vertebrate retina is a dense array of neurons lining the back of the eyeball. It consists of five layers of cells. The outermost layer consists of the rods and cones. The innermost layer consists of the ganglion cells, which send their axons in the optic nerve to the brain. Between the photoreceptors and the ganglion cells are neurons that process the information from the photoreceptors. Review Figure 45.24
- The area of the retina that receives light from the center of the visual field, the fovea, has the greatest density of photoreceptors. In humans the fovea contains almost exclusively cone cells, which are responsible for color vision but are not very sensitive in dim light.
- Each ganglion cell is stimulated by light falling on a small circular patch of photoreceptors called a receptive field. Receptive fields have a center and a surround, which have opposing effects on the ganglion cell. If the center is excitatory, the surround is inhibitory, and vice versa. Review Figure 45.25

Sensory Worlds Beyond Our Experience

- Many animals have sensory abilities that we do not share. Bats echolocate, insects see ultraviolet radiation, pit vipers "see" infrared radiation, and fish sense electric fields.

For Discussion

1. Compare and contrast the functioning of olfactory receptors and photoreceptors. How do these sensory cells enable the CNS to discriminate between an apple and an orange?
2. Amplification of signal is an important feature of sensory systems. Compare mechanisms of amplification in olfactory, visual, and auditory systems.
3. If you were blindfolded and placed in a wheelchair, how would you know if you were being pushed forward or backward?
4. Describe and contrast two sensory systems that enable animals to "see" in the dark. What problems or limitations are inherent in these systems in comparison with vision?

5. Communication is the transfer of information from one animal to another. Animals can use visual, olfactory, tactile, and auditory signals to communicate. From what you know about these sensory systems, discuss the relative advantages and disadvantages of these systems for communication.



The Mammalian Nervous System: Structure and Higher Functions



Phineas Gage was an industrious, responsible, considerate young man. He was 25 years old, working as a railroad construction foreman. He had the respect of his men, and he looked out for them to the extent that he took on himself the most dangerous tasks associated with blasting the rocks in the path of the railroad.

Late one afternoon the last hole had been drilled for the day. Gage poured blasting powder into the hole and tamped it with a meter-long, 3-cm wide iron rod. The tamping iron hit the side of the hole, struck a spark, and ignited the powder. The explosion shot the rod out of the hole like a bullet. It struck Phineas below his left eye, penetrated his skull, passed through the part of his brain behind his forehead, and exited out the top of his head. Was this the end of Phineas Gage?

Gage regained consciousness within minutes and could speak. He was taken to a hotel, where a physician dressed his wounds, but the doctor could do little else. Infections were a problem, but Gage's senses and memory were intact. In 3 weeks he left his bed, but he did not return to his work at the railroad. The recovered Phineas Gage was quarrelsome, bad-tempered, lazy, and irresponsible. He was impatient and obstinate, and he used profane language, which he had never done before.

The body of Phineas Gage survived the accident, but he was an entirely different person. He spent the rest of his days as a drifter, earning money by telling his story, exhibiting his scars and his tamping iron. If you are in Cambridge, Massachusetts, you can pay him a visit. His skull, death mask, and the tamping iron are on display in the Museum of the Medical College of Harvard University.

The sad story of Phineas Gage reveals that the essence of individuality and personality resides in the brain. What is this miraculous organ, and what does it do? The human brain weighs about 1.5 kg, is mostly water, and has the consistency of custard. Yet the complexity of this small mass of tissue exceeds that of any other known matter. The work of the brain is to process and store information and to control the physiology and behavior of the body. The brain is con-

An Unintentional Experiment

In a nineteenth-century railroad construction accident, an explosion blew a tamping iron through the brain of Phineas Gage. Unbelievably, Gage survived, but his personality was radically changed. This drawing of Gage's skull was made at the time of his death.

stantly receiving, integrating, and interpreting information from all the senses. To respond to that information, it generates commands to the muscles and organs of the body.

The unit of function of the brain is the neuron. The human brain consists of about 10 billion neurons, which account for its ability to handle vast amounts of information. In the previous two chapters we learned about the cellular properties of neurons: how they generate and conduct action potentials, how they transduce sensory information, how they communicate with each other at synapses, and how information is integrated at synapses. In this chapter we take on the challenge of understanding the functions of the human nervous system in terms of these cellular mechanisms.

The Nervous System: Structure, Function, and Information Flow

The human nervous system is more than the brain. The brain and spinal cord together constitute the central nervous system (CNS). Information is brought to and from the



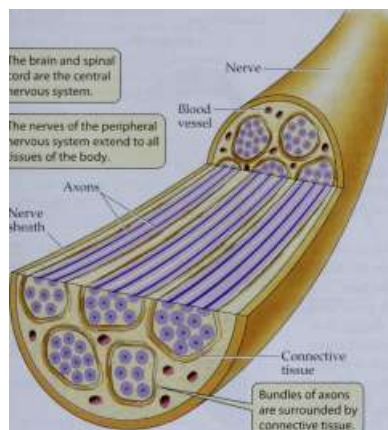
I The brain and spinal cord are the central nervous system.

Nerve -

The nerves of the peripheral nervous system extend to all tissues of the body.

Blood vessel

Axons



Nerve sheath

Bundles of axons are surrounded by connective tissue.

46.1 Anatomy of the Human Nervous System

(a) Information is communicated between the central nervous system and the other tissues of the body through the peripheral nervous system. (fc>) A nerve contains the axons of many neurons. Some of these neurons conduct information to and others from the central nervous system.

THE MAMMALIAN NERVOUS SYSTEM 815

cal regulation (for example, blood pressure, deep body temperature, blood oxygen supply).

The efferent portion of the peripheral nervous system carries information from the CNS to the muscles and glands of the body. Efferent pathways can be divided into a voluntary division, which executes our conscious movements, and an involuntary, or autonomic, division, which controls physiological functions.

In addition to neural information, the CNS receives chemical information in the form of hormones circulating in the blood. Neurohormones released by neurons into the extracellular fluids of the brain can send chemical information to other neurons in the brain or can leave the brain and enter the circulation. In Chapter 41 we learned of the important role of neurohormones in the control of the anterior pituitary and saw that other neurohormones are released from the posterior pituitary into the circulation. Now we can begin to translate our conceptual scheme of information flow into an anatomical view of the nervous system. It can be difficult to learn the relationships between the different structures of the adult nervous system, but the task is much easier if we begin with the development of the nervous system from a simple tubular structure that forms

Connective tissue

in the embryo.

CNS by means of an enormous network of nerves that make up the peripheral nervous system (Figure 46.1a). The peripheral nervous system reaches every tissue of the body. It connects to the CNS via spinal nerves and cranial nerves. A nerve is a bundle of axons (Figure 46.1b) that carries information about many things simultaneously. It is important to distinguish between the axon of a single neuron and a nerve. Some axons in a nerve may be carrying information to the CNS while other axons in the same nerve are carrying information from the CNS to the organs of the body.

A conceptual diagram of the nervous system traces information flow

The nervous system is an information processing system— a very complex one that handles many tasks simultaneously. It will help to organize our thinking about the nervous system by beginning with a conceptual diagram of information flow (Figure 46.2). We can then plug anatomical and functional details into this general model.

The afferent portion of the peripheral nervous system carries information to the CNS. We are consciously aware of much of the information that moves through these afferent pathways (for example, vision, hearing, temperature, pain, the position of limbs), but we are not consciously aware of other afferent information that is important for physiologi-

Neural afferents bring signals to the CNS.

Neural efferents carry signals away from the CNS.

Y

Neural afferents

Hormones

Eyes, ears, joints, skin, skeletal muscles

Conscious

Unconscious

Body organs

PERIPHERAL

NERVOUS

SYSTEM

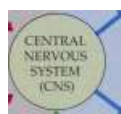
(PNS)

Neurohormones

T

Neural efferents

Skeletal muscles



Voluntary

Autonomic

Glands, smooth muscle, heart muscle

46.2 Organization of the Nervous System

The peripheral nervous system carries information both to and from the central nervous system. The CNS also receives hormonal inputs and produces hormonal outputs.

816 CHAPTER FORTY-SIX

The vertebrate CNS develops from the embryonic neural tube

Early in the development of all vertebrate embryos, a hollow tube of neural tissue forms (see Chapter 43). This neural tube runs the length of the embryo on its dorsal side. At the anterior end of the embryo, the neural tube forms three swellings that become the basic divisions of the brain: the hindbrain, the midbrain, and the forebrain. The rest of the neural tube becomes the spinal cord (Figure 46.3). The cranial and spinal nerves, which make up the peripheral nervous system, sprout from the neural tube and grow throughout the embryo.

Each of the three regions of the embryonic brain develops into several structures in the adult brain. From the hind-brain come the medulla, the pons, and the cerebellum. The medulla is continuous with the spinal cord. The pons is in front of the medulla, and the cerebellum is a dorsal outgrowth of the pons. The medulla and pons contain distinct groups of neurons that are involved in the control of physiological functions such as breathing and circulation or basic motor patterns such as swallowing and vomiting. All neural information traveling between the spinal cord and higher brain areas must pass through the pons and the medulla.

The cerebellum is like the conductor of an orchestra; it receives "copies" of the commands going to the muscles from higher brain areas, and it receives information coming up the spinal cord from the joints and muscles. Thus it can compare the motor "score" with the actual behavior of the muscles and refine the motor commands.

From the embryonic midbrain come structures that process aspects of visual and auditory information. In addition, all information traveling between higher brain areas and the spinal cord must pass through the midbrain.

The embryonic forebrain develops a central region called the diencephalon and a surrounding structure called the telencephalon. The diencephalon is the core of the forebrain and consists of an upper structure called the thalamus and a lower structure called the hypothalamus. The thalamus is the final relay station for sensory information going to the telencephalon, and the hypothalamus is responsible for the regulation of many physiological functions and biological drives.

The telencephalon consists of two cerebral hemispheres, left and right (also referred to as the cerebrum). In humans, the telencephalon is by far the largest part of the brain and plays major roles in sensory perception, learning, memory, and conscious behavior.

Understanding the relationships among the many structures of the complex adult brain is a little easier if you keep this linear organization of the neural axis in mind: Communication between the spinal cord and the telencephalon travels through the medulla, pons, midbrain, and diencephalon. The medulla, pons, and midbrain are referred to collectively as the brain stem. In general, more primitive and autonomic functions are localized farther down this neural axis, while more complex and evolutionary advanced functions are found higher on the axis.

The stretched-out neural tube, viewed from above, shows three swellings that will form the adult brain.

Lateral views

Neural tube



25 days



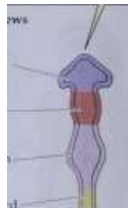
Dorsal views

Forebrain

Midbrain

Hindbrain

Spinal cord



25 days

35 days

Forebrain Midbrain

Hindbrain

Spinal cord

The forebrain develops into two major divisions.



40 days



Telencephalon

Diencephalon M

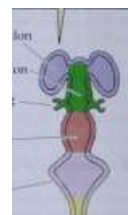
Developing

eve

Midbrain Hindbrain

Cerebral hemisphere

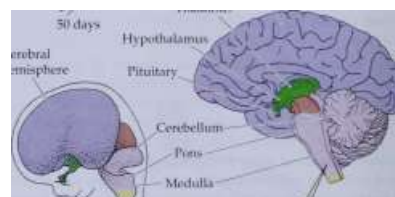
Thalamus



40 days

50 days

Cerebral hemisphere



100 days

The hindbrain develops into three major divisions: the cerebellum, pons, and medulla.

46.3 Development of the Human Nervous System

Three swellings at the anterior end of the hollow neural tube in early vertebrate embryos develop into the parts of the adult brain. The final view is an adult human brain section cut in half through the midline.

As we go up the vertebrate phylogenetic scale from fish to mammals, the telencephalon increases in size, complexity, and importance. The forebrain dominates the nervous systems of mammals, and damage to this region results in severe impairment of sensory, motor, or cognitive functions, and even coma. In contrast, a shark with its telencephalon removed can swim almost normally.

46.4 The Spinal Cord Processes Information

Sensory information (afferent) enters through the dorsal horns (blue pathway), and motor output (efferent) leaves via the ventral horns (orange and red pathways). The extensor component of the knee-jerk response is a monosynaptic reflex circuit, but the flexor inhibition component involves a spinal interneuron (black).

Gray matter

White matter

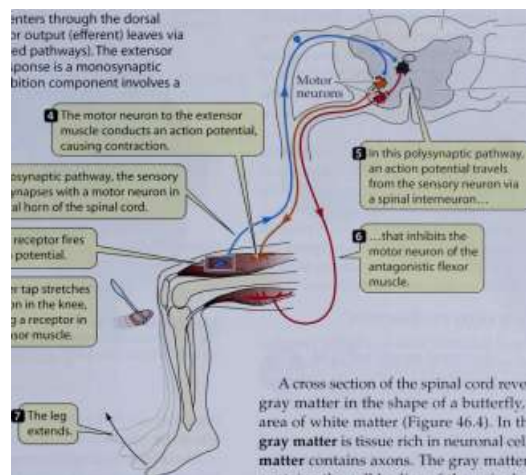
O The motor neuron to the extensor muscle conducts an action potential causing contraction.

Q In a monosynaptic pathway, the sensory neuron synapses with a motor neuron in the ventral horn of the spinal cord.

n this polysynaptic pathway, an action potential travels from the sensory neuron via a spinal interneuron...

f

LVPu A hammer tap stretches the tendon in the knee, stretching a receptor in the extensor muscle.



Dorsal root (afferent nerves)

Dorsal horn

Ventral horn Ventral root (efferent nerves)

A stretch receptor fires an action potential

...that inhibits the motor neuron of the antagonistic flexor muscle.

Functional Subsystems of the Nervous System

When we talk about the development of the nervous system, we describe it in terms of anatomically distinct structures. However, the nervous system is always engaged in many tasks at the same time—a phenomenon called parallel processing of information. Any one task usually involves many different anatomical structures. Understanding the nervous system is made simpler if we recognize its functional subsystems, such as the spinal cord, reticular system, limbic system, and cerebrum. Any one anatomical structure may play roles in several functional subsystems.

AFPA The spinal cord receives and processes information from the body

The spinal cord conducts information in both directions between the brain and the organs of the body. It also integrates a great deal of the information coming from the peripheral nervous system and responds to that information by issuing motor commands.

The conversion of afferent to efferent information in the spinal cord without participation of the brain is called a spinal reflex. The simplest type of spinal reflex involves only two neurons and one synapse and is therefore called a monosynaptic reflex. An example is the knee-jerk reflex, which your physician checks by tapping just below your knee with a small mallet. We can diagram the wiring of a monosynaptic reflex by following the flow of information through the spinal cord.

A cross section of the spinal cord reveals a central area of gray matter in the shape of a butterfly, surrounded by an area of white matter (Figure 46.4). In the nervous system, gray matter is tissue rich in neuronal cell bodies, and white matter contains axons. The gray matter of the spinal cord contains the cell bodies of the spinal neurons; the white matter contains axons.

the axons that conduct information up and down the spinal cord. Spinal nerves leave the spinal cord at regular intervals on each side. Each spinal nerve has two roots connecting to the gray matter—one connecting with the dorsal horn, the other with the ventral horn. Each spinal nerve carries both afferent and efferent information. The afferent axons enter the spinal cord through the dorsal roots and the efferent axons leave the spinal cord through the ventral roots.

In the case of the knee-jerk reflex, sensory information comes from stretch receptors in the leg muscle that is suddenly stretched when the mallet strikes the tendon that runs over the knee. Each stretch receptor initiates action potentials that are conducted by the axon of a sensory neuron in through the dorsal horn of the spinal cord and all the way to the ventral horn. In the ventral horn, the sensory neuron synapses with motor neurons, causing them to fire action potentials that are then conducted back to the leg extensor muscle, causing it to contract. The function of this simple circuit is to sense an increased load on the limb, and to cause the muscle to increase its strength of contraction to compensate for the added load.

Most spinal circuits are more complex than this monosynaptic reflex. We can demonstrate that by building on the circuit we have just traced. Limb movement is controlled by antagonistic sets of muscles—muscles that work against each other. When one member of an antagonistic set of muscles contracts, it bends or flexes the limb; it is therefore called a flexor. The antagonist to this muscle straightens or extends the limb and is called an extensor. For a limb to move, one muscle of the pair must relax while the other

818 CHAPTER FORTY-SIX

contracts. 1 hus sensor) input that activates the motor neuron of one muscle also inhibits its antagonist. This coordination is achieved b\ an interneuron, which makes an inhibitor sj napse onto the motor neuron oi the antagonistic muscle (six- Figure 46.4). I bus the reciprocal inhibition of antagonistic muscles involves an interneuron between the sensory cell and the motor neuron and therefore at least two s) rtapses.

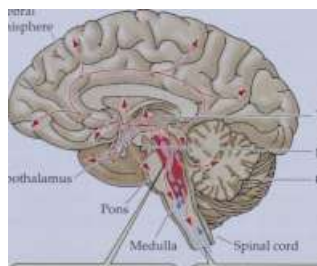
Information entering the dorsal horn is also transmitted b\ axons up the spinal cord to the brain. We are aware of the mallet hitting the knee, but the reflex response actually begins before that information registers in our consciousness. A great deal of information processing takes place in the spinal cord without any input from the brain. Spinal circuits can even generate repetitive motor patterns such as those o\ walking without commands from the brain.

The reticular system alerts the forebrain

The reticular system of the brain stem is a highly complex network of neuronal axons and dendrites. Within the reticular system are many discrete groups of neurons. Such an anatomically distinct group of neurons in the CNS is called a nucleus.

The reticular system is distributed through the core of the medulla, pons, and midbrain (Figure 46.5). Afferent information coming up the neural axis passes through the reticular system, where many connections are made to neurons involved in controlling many functions of the body. Information from joints and muscles, for example, is directed to nuclei in the pons and cerebellum that are involved in balance and coordination, whereas information from pain receptors is directed to nuclei in the brain stem that control sensitivity to pain. This information continues up the neural axis to the forebrain, where it results in conscious sensa-

Cerebral hemisph



Hypothalamus

Thalamus

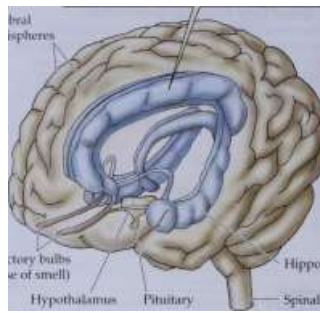
Midbrain Cerebellum

The reticular system contains groups of cells whose axons travel throughout the forebrain and control sleep and wakefulness.

Sensory pathways from the spinal cord make many connections in the reticular system.

Structures deep within the cerebral hemispheres and surrounding the hypothalamus control aspects of motivation, drives, emotions, and memory.

Cerebral hemispheres



Olfactory bulbs (sense of smell)

Hypothalamus Pituitary

Hippocampus Spinal cord

46.5 The Reticular System

Neuronal activity within the reticular system controls levels of arousal in the nervous system.



46.6 The Limbic System

The evolutionary primitive parts of the forebrain (shown in blue) are referred to as the limbic system.

Information that can be localized to the specific sites in the body where the information originated.

The information routed through the reticular system also influences the level of arousal of the nervous system. Nuclei in the reticular system are involved in the control of sleep and waking. High levels of activity in the reticular system influence these nuclei to maintain the brain in a waking condition; low levels of activity enable sleep. Because of the alerting function of the reticular core of the brain stem, it has been called the reticular activating system.

If the brain stem of a person is damaged at midbrain or higher levels, and the alerting action of the reticular system cannot reach the forebrain, the person loses the ability to be in a conscious, waking state and becomes comatose. Damage to the brain stem or the spinal cord below the reticular system does not interfere with the ascending alerting actions of the reticular system and leaves the person with normal patterns of sleep and waking, although it can cause lack of sensation (paresthesia) and loss of motor function (paralysis).

The limbic system supports basic functions of the forebrain

The telencephalon of fishes, amphibians, and reptiles consists of only a few structures surrounding the diencephalon. In birds and mammals, these primitive forebrain structures are completely covered by the evolutionary more recent elaborations of the telencephalon called the neocortex, but they still have important functions. These primitive parts of the forebrain are collectively referred to as the limbic system (Figure 46.6).

The limbic system is responsible for basic physiological drives, instincts, and emotions. Within the limbic system are areas that when stimulated with small electric currents can cause intense sensations of pleasure, pain, or rage. If a

(a)

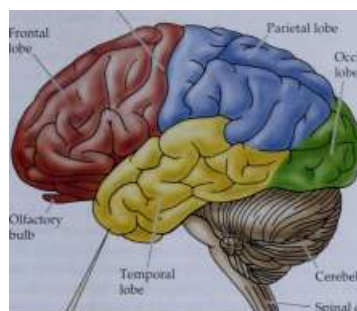
(b)

Central sulcus

Frontal lobe

Parietal lobe

Occipital lobe



Cerebellum Spinal cord

The highly convoluted halves of the cerebrum, viewed here from the left side, cover most of the other structures of the brain.

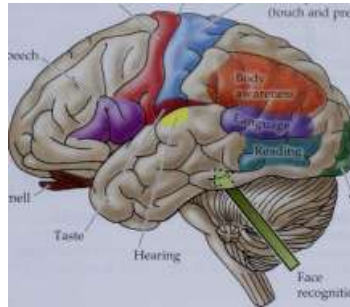
Primary motor cortex (motor control)

Central Primary somato-

sulcus sensory cortex

(touch and pressure)

Speech



Smell

Taste

Vision

Face

recognition

rn



46.7 The Human Cerebrum

(a) Each cerebral hemisphere is divided into four lobes. (6) Different functions are localized in particular areas of the cerebral lobes.

rat is given the opportunity to stimulate its own pleasure centers by pressing a switch, it will ignore food, water, and even sex, pushing the switch until it is exhausted. Pleasure and pain centers in the limbic system are believed to play roles in learning and in physiological drives.

One part of the limbic system, the hippocampus, is necessary in humans for the transfer of short-term memory to long-term memory. If you are told a new telephone number, you may be able to hold it in short-term memory for a few minutes, but within half an hour it is forgotten unless you make a real effort to remember it. The phenomenon of remembering something for more than a few minutes requires its transfer to long-term memory.

Regions of the cerebrum interact to produce consciousness and control behavior

The cerebral hemispheres are the dominant structures in the mammalian brain. In humans they are so large that they cover all other parts of the brain except the cerebellum (Figure 46.7). A sheet of gray matter called the cerebral cortex covers each cerebral hemisphere. The cortex is about 4 mm thick and covers a total surface area over both hemispheres of 1 square meter. The cerebral cortex is convoluted, or folded, into ridges called gyri and valleys called sulci. These convolutions allow it to fit into the skull, which is of limited size and volume. Under the cerebral cortex is white matter, made up of the axons that connect the cell bodies in the cortex with one another. They also connect with other areas of the brain.

Different regions of the cerebral cortex have specific functions. Some of those functions are easily defined, such as receiving and processing sensory information, but most of the cortex is involved in higher-order information pro-

cessing that is less easy to define. These latter areas are given the general name of association cortex.

To understand the cerebral cortex, it helps to have an anatomical road map. As viewed from the left side, a left cerebral hemisphere looks like a boxing glove for the right hand with the fingers pointing forward, the thumb pointing out, and the wrist at the rear (see Figure 46.7a). The "thumb" area is the temporal lobe, the fingers the frontal lobe, the back of the hand the parietal lobe, and the wrist the occipital lobe. A mirror image of this arrangement characterizes the right cerebral hemisphere. Let's look at each lobe of the cerebrum separately.

the temporal lobe. The upper region of the temporal lobe receives and processes auditory information. The association areas

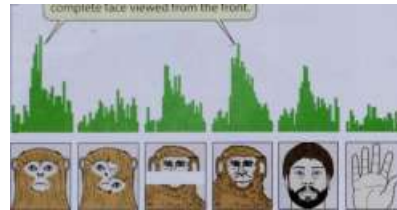
of the temporal lobe are involved in the recognition, identification, and naming of objects. Damage to the temporal lobe results in disorders called agnosias in which the individual is aware of a stimulus but cannot identify it. Some of these deficits can be quite specific.

Damage to one area of the temporal lobe results in the inability to recognize faces. Even old acquaintances cannot be identified by facial features, although they may be identified by other attributes such as voice, body features, and characteristic style of walking. Using monkeys, it has been possible to record from neurons in this region that respond selectively to faces in general. These neurons do not respond to other stimuli in the visual field, and their responsiveness decreases if some of the features of the face are missing or appear in inappropriate locations (Figure 46.8). Damage to other association areas of the temporal lobe causes deficits in understanding spoken language, even though speaking, reading, and writing abilities may be intact.

820 CHAPTER FORTY-SIX

This neuron responds maximally to a complete face viewed from the front.

Firing rate of neuron



jJkIU

46.8 Neurons in One Region of the Temporal Lobe Respond to Faces

The traces represent the firing rate of a neuron in the temporal lobe of a monkey in response to the pictures shown below them.

the frontal lobe. A strip of the frontal lobe cortex just in front of the central sulcus is called the primary motor cortex (see Figure 46.7b). The neurons in this region have axons that project to muscles in specific parts of the body. The parts of the body can be mapped onto the primary motor cortex, from the head region on the lower side to the lower part of the body at the top. Areas with fine motor control, such as the face and hands, have the greatest representation (Figure 46.9). If a neuron in the primary motor cortex is electrically stimulated, the response is the twitch of a muscle, but not a coordinated, complex behavior.

The association functions of the frontal lobe are diverse and are best described as having to do with planning. The story of Phineas Gage at the beginning of this chapter demonstrates the effects of damage to these association areas. People with such deficits have drastic alterations of personality because they cannot create an accurate view of themselves in the context of the world around them and cannot plan for future events.

the parietal lobe. The frontal and parietal lobes are separated by a deep valley called the central sulcus. The strip of parietal lobe cortex just behind the central sulcus is the primary somatosensory cortex (see Figure 46.7b). This area receives information through the thalamus about touch and pressure sensations.

The whole body surface is represented in the primary somatosensory cortex—the head at the bottom and the legs at the top (see Figure 46.9). Areas of the body that have lots of sensory neurons and are capable of making fine distinctions in touch (such as the lips and the fingers) have disproportionately large representation. If a

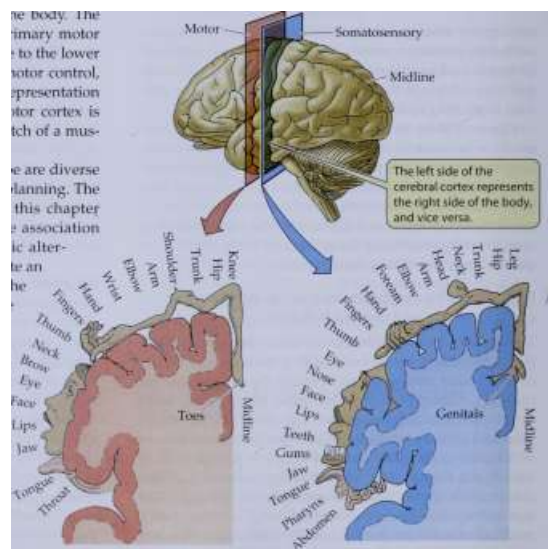
very small area of the primary somatosensory cortex is stimulated electrically, the subject reports feeling specific sensations, such as touch, from a very localized part of the body.

A major association function of the parietal lobe is attending to complex stimuli. Damage to the right parietal lobe causes a condition called contralateral neglect syndrome, in which the individual tends to ignore stimuli from the left side of the body or the left visual field. Such individuals have difficulty performing complex tasks such as dressing the left side of the body; an afflicted man may not be able to shave the left side of his face. When asked to copy simple drawings, a person who exhibits this syndrome can do well with the right side of the drawing, but not the left (Figure 46.10). The parietal cortex is not symmetrical with respect to its role in attention. Damage to the left parietal cortex does not cause neglect of the right side of the body. We will see similar asymmetries in cortical function when we discuss language.

Motor

Somatosensory

Midline



Motor cortex

Somatosensory cortex

46.9 The Body Is Represented in the Primary Motor Cortex and the Primary Somatosensory Cortex

Cross sections through the primary motor and primary somatosensory cortices can be represented as maps of the human body. Body parts are shown in relation to the brain area devoted to them.

THE MAMMALIAN NERVOUS SYSTEM

821

Model



Patient's copy



46.10 Contralateral Neglect Syndrome

A person with damage to the right parietal association cortex will neglect the left side of a drawing when asked to copy a model.

the occipital lobe. The occipital lobes receive and process visual information. We'll learn more about the details of that process later in this chapter. The association areas of the occipital cortex are essential for making sense out of the visual world and translating visual experience into language. Some deficits resulting from damage to association areas of the occipital cortex are quite specific. In one case, a woman with limited damage was unable to see motion. Her vision was intact, but she could see a waterfall only as a still image, and a car approaching only as a series of scenes of a stationary object at different distances.

The cerebrum has increased in size and complexity

As mentioned earlier, the size of the telencephalon relative to the rest of the brain increases substantially as we go from fishes to amphibians, to reptiles, to birds and mammals. Even when we consider only mammals, the cerebral cortex increases in size and complexity when we compare animals such as rodents, whose behavioral repertoires are relatively simple, with animals such as primates that have much more complex behavior.

The most dramatic increase in the size of the cerebral cortex took place during the last several million years of human evolution. The incredible intellectual capacities of *Homo sapiens* are associated with enlargement of the cerebral cortex. Humans do not have the largest brains in the animal kingdom; elephants, whales, and porpoises have larger brains in terms of mass. If we compare brain size to body size, however, humans and dolphins top the list. Humans have the largest ratio of brain size to body size, and they have the most highly developed cerebral cortex. Another feature of the cerebral cortex that reflects increasing behavioral and intellectual capabilities is the ratio of association cortex to primary somatosensory and

motor cortexes. Humans have the largest relative amount of association cortex.

Information Processing by Neuronal Networks

In Chapter 44 we learned how neurons interact to process information. A goal of neurobiology is to understand the complex functions of the nervous system in terms of the properties of neurons and synapses between them. We will

use two subsystems as examples to show how the functions of the nervous system can be understood in terms of neuronal networks. The first example, the autonomic nervous system, consists of efferent pathways. The second, the visual system, consists of afferent and integrative pathways. Techniques that have allowed neurobiologists to trace neuronal connections, chemically characterize synapses, and record the activities of single cells and groups of cells have advanced our understanding of how certain subsystems of the nervous system work.

The autonomic nervous system controls organs and organ systems

The autonomic nervous system is divided into two parts: the sympathetic and parasympathetic divisions. These two divisions work in opposition to each other in their effects on most organs, one causing an increase in activity and the other causing a decrease. The best-known functions of the autonomic nervous system are those of the sympathetic division called the "fight-or-flight" mechanisms, which increase heart rate, blood pressure, and cardiac output and prepare the body for emergencies (see Chapter 41). In contrast, the parasympathetic division slows the heart and lowers blood pressure.

It is tempting to think of the sympathetic division as the one that speeds things up and the parasympathetic division as the one that slows things down, but that is not always a correct distinction. The sympathetic division slows the digestive system and the parasympathetic division accelerates it. The two divisions of the autonomic nervous system are easily distinguished from each other by their anatomy, their neurotransmitters, and their actions (Figure 46.11).

Both divisions of the autonomic nervous system are efferent pathways. Each autonomic efferent pathway begins with a neuron that has its cell body in the brain stem or spinal cord and uses acetylcholine as its neurotransmitter. These cells are called preganglionic neurons because the second neuron in the pathway with which they synapse resides in a ganglion, a collection of neuronal cell bodies that is outside of the CNS). The second neuron is called a post-

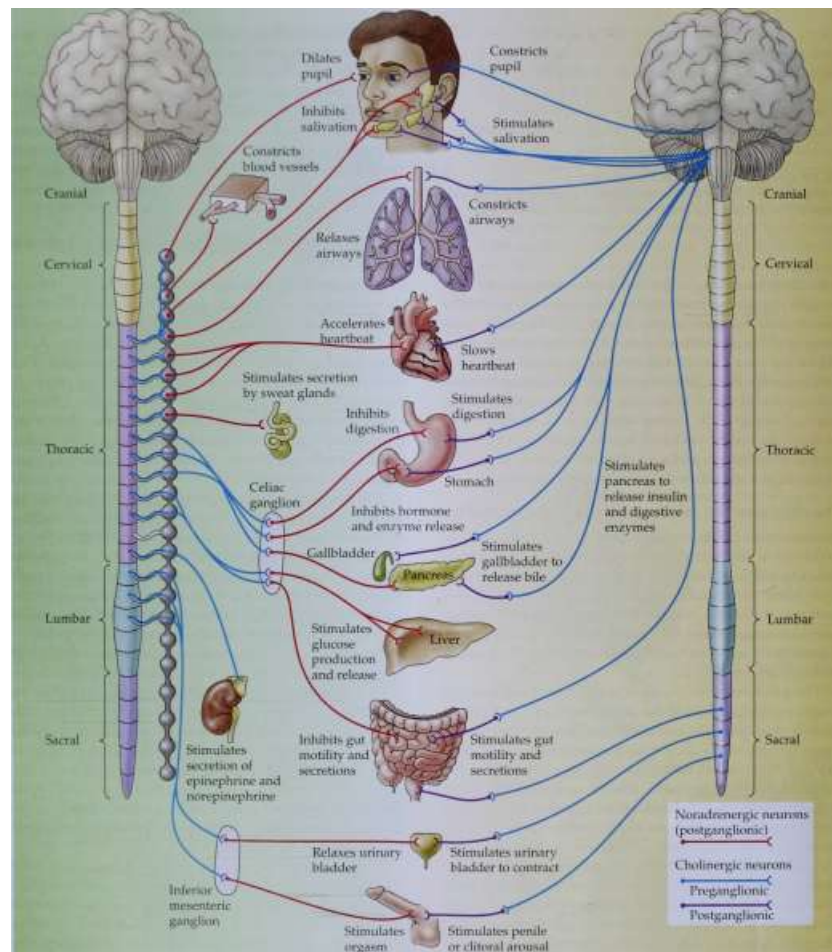
ganglionic neuron

because its axon extends out from the ganglion. The axons of the postganglionic neurons end on the cells of the target organs.

The postganglionic neurons of the sympathetic division use norepinephrine as a neurotransmitter; those of the parasympathetic division use acetylcholine. In organs that receive both sympathetic and parasympathetic input, the target cells respond in opposite ways to norepinephrine and to acetylcholine. A region of the heart called the pacemaker, which generates the heartbeat, is an example. Stimulating the sympathetic nerve to the heart or dripping norepinephrine onto the pacemaker region depolarizes the pacemaker cells, increases their firing rate, and causes the heart to beat faster. Stimulating the parasympathetic nerve to the heart or dripping acetylcholine onto the pacemaker region hyperpolarizes the pacemaker cells, decreases their firing rate, and causes the heart to beat slower. In contrast,

Sympathetic division

Parasympathetic division



46.11 Organization of the Autonomic Nervous System

The autonomic nervous system is divided into the sympathetic and parasympathetic divisions, which work in opposition to each other in their effects on most organs (one causing an increase and the other a decrease in activity).

in the digestive tract, norepinephrine hyperpolarizes muscle cells, which slows digestion, and acetylcholine depolarizes muscle cells, which accelerates digestion.

The sympathetic and parasympathetic divisions of the autonomic nervous system can also be distinguished by anatomy (see Figure 46.11). The preganglionic neurons of the parasympathetic division come from the brain stem and the last segment of the spinal cord. The preganglionic neurons of the sympathetic division come from the upper regions of the spinal cord below the neck—the thoracic and

lumbar regions. The ganglia of the sympathetic division are mostly lined up in two chains, one on either side of the spinal cord. The parasympathetic ganglia are close to—sometimes sitting on—the target organs.

The autonomic nervous system is an important link between the CNS and many physiological functions of the body. Its control of diverse organs and tissues is crucial to homeostasis. In spite of its complexity, work by neurobiologists and physiologists over many decades has made it possible to understand its functions in terms of neuronal properties and circuits. In Chapter 49, for example, we will see how information from pressure receptors in the blood vessels is transmitted to the CNS, where it produces autonomic signals that control the rate of the heartbeat.

Neurons and circuits in the occipital cortex integrate visual information

In Chapter 45 we learned that the information conveyed to the brain in the optic nerve consists of action potentials that are stimulated by light falling on small circular areas of the retina called receptive fields. A receptive field contains many photoreceptor cells connected together in a circuit in such a way that the signals they produce are integrated and transmitted to the brain by a single retinal ganglion cell. The axon of each ganglion cell travels to the brain in the optic nerve. How does the brain construct visual images from information about circular patches of light falling on the retina?

Information from the retina is transmitted through the optic nerve to a relay station in the thalamus, and then to the brain's visual processing area, in the occipital cortex at the back of the cerebral hemispheres (see Figure 46.7b). David Hubel and Torsten Wiesel of Harvard University studied the activity of neurons in this visual cortex. They recorded the activities of single cells in the brains of living animals while they stimulated the animals' retinas with spots and bars of light. They found that cells in the visual cortex, like retinal ganglion cells, have receptive fields—specific areas of the retina that, when stimulated by light, influence the rate at which the action potentials.

Cells in the visual cortex, however, have receptive fields that differ from the simple circular receptive fields of retinal ganglion cells. Cortical cells called simple cells are maximally stimulated by bars of light that have specific orientations. Simple cells

probably receive input from several ganglion cells whose circular receptive fields are lined up in a row.

Complex cells in the visual cortex are also maximally stimulated by a bar of light with a particular orientation, but

46.12 Receptive Fields of Cells in the Visual Cortex

Cells in the visual cortex respond to specific patterns of light falling on the retina. Ganglion cells that project information about circular receptive fields converge on simple cells in the cortex in such a way that the simple cells have linear receptive fields. Simple cells project to complex cells in such a way that the complex cells can respond to linear stimuli falling on different areas of the retina.

the bar may fall anywhere on a large area of retina described as that cell's receptive field. Complex cells receive input from several simple cells that share a certain stimulus orientation, but have receptive fields in different places on the retina (Figure 46.12). Some complex cells respond most strongly when the bar of light moves in a particular direction.

The concept that emerges from these experiments is that the brain assembles a mental image of the visual world by analyzing edges of patterns of light falling on the retina.

EXPERIMENT

Question: How do cells in the visual cortex respond to patterns of light falling on the retina?

METHOD

The bar of light moves across the screen.

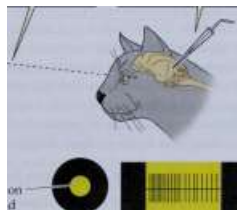
A moving bar of light stimulates receptive fields in the retina.

As the cat views the screen, the electrode records the cells in the occipital cortex.



RESULTS

On-center ganglion cell receptive field

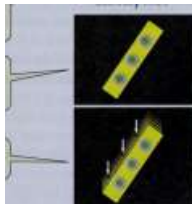


Retinal ganglion cells that are aligned project to thalamic relay cells.

On-center ganglion cell response

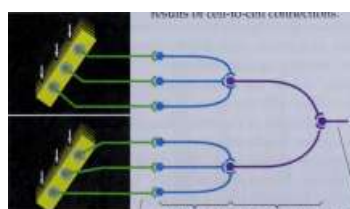
Simple cells in the cortex respond to a static bar of light at a particular angle and location.

Complex cells in the cortex respond to a moving bar of light



INTERPRETATION This model would explain the

results of cell-to-cell connections.

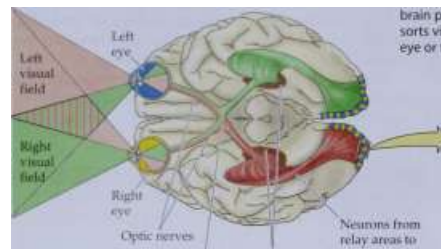


Relay cells Simple cells Complex cells Retinal ganglion cells in thalamus in cortex in cortex

Conclusion: Cells in the retina, thalamus, and cortex are connected in ways to respond to specific patterns of light.

824 CHAPTER FORTY-SIX

illumination in brain (viewed from underneath)



46.13 The Anatomy of Binocular Vision

Each eye transmits information to both sides of the brain; however, the right brain processes all information from the left visual field, and the left brain processes all information from the right visual field. The visual cortex sorts visual field information according to whether it comes from the right eye or the left eye.

Optic chiasm Relay areas in thalamus

Neurons from relay areas to visual cortex

This analysis is conducted in a massively parallel fashion. Each retina sends a million axons to the brain, but there are hundreds of millions of neurons in the visual cortex. Each bit of information from a retinal ganglion cell is received by hundreds of cortical cells, each responsive to a different combination of orientation, position, and even movement of contrasting lines in the pattern of light falling on the retina.

Cortical cells receive input from both eyes

How do we see objects in three dimensions? The quick answer is that our two eyes see overlapping, yet slightly different, visual fields; that is, we have binocular vision. Turn a typical conical flowerpot upside down and look down at it so that the bottom of the pot is exactly in the center of your overall field of vision. You see the bottom of the pot, and you see equal amounts of the sides and rim of the pot as concentric circles. Now, if you close your left eye, you see more of the right side and right rim of the pot. With your right eye closed, you see more of the left side and left rim of the pot. The discrepancies in the information coming from your two eyes are interpreted by the brain to provide information about the depth and the three-dimensional shape of the flowerpot. If you are blind in one eye, you have great difficulty discriminating distances. Animals whose eyes are on the sides of their heads have nonoverlapping fields of vision and, as a result, poor depth vision, but they can see predators creeping up from behind.

The story of how the brain integrates information from two eyes begins with the paths of the optic nerves. If you look at the underside of the brain, the optic nerves from the two eyes appear to join together just under the hypothalamus and then separate again. The place where they join is called the optic chiasm (Figure 46.13). Axons from the half of each retina closest to your nose cross in the optic chiasm and go to the opposite side of your brain. The axons from the other half of each retina go to the same side of the brain.

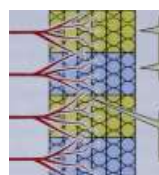
The result of this division of axons in the optic chiasm is that all visual information from your left visual field

Right eye

Left eye

Right eye

Left eye



The visual cortex is organized in columns that receive input from the right eye (yellow) and the left eye (blue).

Binocular cells at the borders of columns receive input from both the right and left eyes.

(everything left of straight ahead) goes to the right side of your brain, as shown in red in Figure 46.13. All visual information from your right visual field goes to the left side of your brain, as indicated in green in the figure. Both eyes transmit information about a specific spot in your right visual field, for example, to the same place in the left visual cortex. How are the

two sources of information integrated?

Cells in the visual cortex are organized in columns. These columns alternate: left eye, right eye, left eye, right eye, and so on. Cells closest to the border between two columns receive input from both eyes and are therefore called binocular cells. Binocular cells interpret distance by measuring the disparity between where the same stimulus falls on the two retinas.

What is disparity? Hold your finger out in front of you and look at it, closing one eye and then the other. Your finger appears to jump back and forth because its image falls on a different position on each retina. Repeat the exercise with an object at a distance. It doesn't appear to jump back and forth as much because there is less disparity in the positions of the image on the two retinas. Certain binocular cells respond optimally to a stimulus falling on both retinas with a particular disparity. Which set of binocular cells is stimulated depends on how far away the stimulus is.

When we look at something, we can detect its shape, color, depth, and movement. Where does all this information come together? Is there a single cell that fires only when a red sports car drives by? Probably not. A specific visual experience comes from simultaneous activity in a large collection of cells. In addition, most visual experiences are enhanced by information from the other senses and from memory as well. This realization helps explain why about 75 percent of the cerebral cortex is association cortex.

Understanding Higher Brain Functions in Cellular Terms

Very few functions of the nervous system have been worked out to the point of identifying the underlying neuronal networks. The processes responsible for the higher brain functions discussed in the remaining pages are unde-

THE MAMMALIAN NERVOUS SYSTEM 825

niably complex. Nevertheless, neurobiologists, using a wide range of techniques, are making considerable progress in understanding some of the mechanisms involved. The following discussion presents several complex aspects of brain and behavior that present challenges to neurobiologists: sleep and dreaming, learning and memory, language use, and consciousness.

Sleeping and dreaming involve electrical patterns in the cerebrum

A dominant feature of our behavior is the daily cycle of sleep and waking. All birds and mammals, and probably all other vertebrates, sleep. We spend OQ£-thkd_aJLmu4ives sleepjn^j^etLwe_dojiot know why or how.

We do know, however, that we need to sleep. Loss of sleep impairs alertness and performance. Most people in our society—certainly most college students—are chronically sleep-d epriv ed. Large numbers of accidents and serious mistakes that endanger lives can be attributed to impaired alertness due tosleep.loss. Yet insomnia (difficulty in falling or staying asleep) is one of the most common medical complaints. To discover ways of dealing with these problems, it is important to learn more about the neural control of sleep.

the electroencephalogram. A common tool of sleep researchers is the electroencephalogram jCEEG^. To record an EEG, electrodes are placed at differemTocations on the scalp, and changes in the electric potential differences between electrodes are recorded through time. These electric potential differences reflect the electrical activity of the neurons in the brain regions under the electrodes, primarily regions of the cerebral corte x. Pens writing on a moving chart are used to record the patterns of electric potential differences between electrodes (Figure 46.14rt,fr). Usually, the

electrical activity of one or more skeletal muscles is also recorded on the chart; this record is called an electromyogram (EMG).

EEG and EMG patterns reveal the transition from being awake to being asleep. They also reveal that there are different states of sleep. In mammals other than humans, two major sleep states are easily distinguished. They are called slow-wave sleep and rapid-eye-movement (REM) sleep. In humans, we characterize sleep statesasTibn-kXM sleep and REM^sleep. Humaji_ non-REM sleep is divided into four stages. o>nly the two deepest stages are considered true^ko^y^wave_sleep.

When a person falls asleep at night, the first sleep state entered is non-REM^sleep, which progresses from stage 1 to stage_4. Stages 3 and 4 are deep, restorative, slow-wave sleep. After this first episode 6Fhon-REM sleep follows an episode of REM sleep. Throughout the night, we have four or five cycles of non-REM and REM sleep (Figure 46.14c). About JO percent of our sleep is non-REM sleep and 20 percent is REM sleep .

We have vivid dreams and nightmares during REM sleep, which gets its name from the jerky movements of the eyeballs that occur during this state. The most remarkable feature of REM sleep is that in hibitory commands from th e br ain almost completely paralyze the skeletal muscles^. Occasional muscle twitches break through the paralysis, as can be seen in a dog that appears to be trying to run in its sleep. If you look closely at a sleeping dog when its legs and paws are twitching, you will be able to see the rapid eye movements as well. Probably the functionofmuscle paralysis during REM sleep is to prevent the acting out of drea ms^ Sleepwalking, however, occurs during non-REM sleep.

The EEG characterization of sleep raises many questions. Why do we have such very different states of sleep?

(«)



46.14 Patterns of Electrical Activity in the Cerebral Cortex Characterize Stages of Sleep

(a) Electrical activity in the cerebral cortex is detected by electrodes placed on the scalp and recorded on moving chart paper by a polygraph, (b) The resulting record is an electroencephalogram (EEG). (c) During a night, we cycle through the different stages of sleep.

(b) (c)

Awake *f***t\h*w4\hA^fa4j^ Awake r—

REM vH^V-Y^-v\^^ A v y v vV-*v»/Vvv-* / REM

Stage 1 ^^MIHM^^^v^Wk^v^iN^ Stage 1

Stage 2 v-AA^-vAy^v^^/AVV'^^^-^ Stage 2

Stage 3. wf^^riysAN^n^^ Stage 3



10 Time (seconds)

2 3 4 5 Time (hours)

826 CHAPTER FORTY-SIX

\\ h\ does non-REM sleep precede and cycle with REM sleep 1 \\ In do we dream in REM sleep? Why do we have deeper non-REM sleep (stages 3 and 4) earlier in the night and more REM sleep later in the night? The answers to these questions are beginning to be revealed as we improve our understanding of the cells and circuits that underlie sleep.

cellular changes during sleep. There are striking neuropil biological differences between non-REM and REM sleep. Non-REM sleep is characterized by a decrease in the $rp^{pnngjvpe}n^n^{jQa^{TTTTTrT}}11b4^{H4JTrejmd}rprphrafor-tex$. Remember that neurons have a resting membrane potential that is negative (the inside of the cell is negative relative to the outside), and a threshold potential for firing action potentials. Usually the resting potential is below the threshold potential, so the neuron is not firing. When synaptic input causes the plasma membrane to become less negative (depolarized), the cell can reach the threshold potential and fire action potentials.

During waking, several nuclei in the brain stem are continuously active. Many axons from these nuclei extend to the thalamus and the cortex, and the neurotransmitters released by the terminals of these axons are generally depolarizing. Therefore, these broadly distributed neurotransmitters keep the resting potential of the neurons of the thalamus and cortex close to threshold and sensitive to synaptic inputs, and thereby maintain waking.

With the onset of sleep, the activity in these brain stem nuclei decreases, and their terminals in the thalamus and cerebral cortex release less neurotransmitter. With the withdrawal of these depolarizing neurotransmitters, the resting potentials of the cells of the thalamus and cerebral cortex become more negative (hyperpolarized), and the cells are less sensitive to synaptic input. Their processing of information is inhibited, and consciousness is lost.

An interesting neuronal event happens as a result of thalamo-cortical hyperpolarization: The cells begin to fire in bursts. The synchronization of these bursts over broad areas of cerebral cortex results in the EEG slow-wave pattern that characterizes non-REM sleep. Studies of neurons of the thalamus and the cortex using intracellular recording techniques have shown that their hyperpolarization during non-REM sleep is due to increased opening of K^+ channels, and the bursting is due to Ca^{2+} channels that rapidly deactivate and require hyperpolarization to be reactivated. We can therefore explain the EEG pattern of non-REM sleep in terms of the properties of neurons and ion channels.

In addition to the brain stem nuclei that bring on sleep by decreasing their activity and causing general hyperpolarization, a substance that is broadly distributed in the extracellular fluid of the brain has a strong hyperpolarizing influence. This substance is adenosine —part of the molecule ATP, which supplies energy for most cellular processes. When cells cannot maintain an adequate supply of ATP they release adenosine. It has therefore been suggested that increased concentrations

of adenosine in the

brain reflect the depletion of brain energy reserves, and that due to its hyperpolarizing influence, adenosine contributes to sleepiness and to the depth of non-REM sleep. A corollary of this hypothesis is that one function of non-REM sleep could be to restore brain energy reserves. There are many other molecules in the brain that influence sleep. Understanding their role in sleep control will provide new information on the possible functions of sleep.

At the transition from rapid eye movement (REM) sleep, dramatic changes occur. Some of the brain stem nuclei that were inactive during non-REM sleep become active again, causing a general depolarization of cortical neurons. Thus the bursts of firing cease, the slow waves in the EEG disappear, and the EEG resembles that of the waking brain. Because the resting potentials of the neurons return to near threshold levels, the cortex can process information, and vivid dreams occur.

During sleep, however, the brain inhibits both afferent (sensory) and efferent (motor) pathways; therefore the activity in the cortex is unconstrained by its usual sources of information. One example of the effect of this loss of motor output and sensory input is the frequently reported dream in which a person is trying to run but cannot move. We do not know the function of REM sleep, but since a wide variety of mammals have about the same percentage of total sleep time that is REM sleep, it is probably a rather basic, cellular function.

One prominent hypothesis about the functions of sleep is that it is essential for the maintenance and repair of neural connections, and for the neural changes that are involved in learning and memory. However, evidence for such functions is still meager.

Some learning and memory can be localized to specific brain areas

Learning is the modification of behavior by experience. Memory is the ability of the nervous system to retain what is learned and what is experienced. Even very simple animals can learn and remember, but these two abilities are most highly developed in humans.

Consider the amount of information associated with learning a language. The capacity of memory and the rate at which items can be retrieved are remarkable features of the human nervous system. A major challenge in neurobiology is to understand these phenomena in terms of the cells and molecules that make up the brain. Such knowledge could help us find ways to prevent the tragic loss of learning abilities and memory that occurs in the common condition of the elderly called Alzheimer's disease.

learning. Learning that leads to long-term memory and modification of behavior must involve long-lasting synaptic changes. Synaptic changes that last for weeks have been discovered, as we saw in Chapter 44. High-frequency electrical stimulation of certain identifiable circuits of the mammalian hippocampus makes them more sensitive to subsequent stimulation. This phenomenon, called long-term

potentiation (LTP), is an effect of ion channels controlled by the neurotransmitter glutamate. These channels allow both Na^+ and Ca^{2+} ions to enter the neurons, and the increase in intracellular Ca^{2+} activates enzymes that cause modifications in the cell that enhance its responsiveness.

In contrast, continuous, repetitive, low-level stimulation of the hippocampal circuit reduces its responsiveness, a phenomenon that has been called long-term depression (LTD). LTP and LTD have been demonstrated in circuits other than hippocampal circuits, and they may be fundamental cellular or molecular mechanisms involved in learning and memory.

A form of learning that is widespread among animal species is associative learning, in which two unrelated stimuli become linked to the same response. The simplest example of associative learning is the conditioned reflex, discovered by the Russian physiologist Ivan Pavlov. Pavlov was studying the control of digestive functions in dogs and observed that a dog salivates at the sight or smell of food—a simple autonomic reflex. He discovered that if he rang a bell just before food was presented to the dog, after a few trials the dog would salivate at the sound of the bell, even if no food followed. The salivation reflex was conditioned to be associated with the sound of a bell, which normally is unrelated to feeding and digestion.

This simple form of learning has been studied extensively in efforts to understand its underlying neural mechanisms. In a series of studies led by Richard Thompson, the eye-blink reflex of a rabbit in response to a puff of air directed at its eyes was conditioned to be associated with a tone stimulus. After conditioning, the rabbit blinked when presented with just the tone (Figure 46.15). A small and specific area of the cerebellum was discovered to be necessary for this conditioned reflex. Thus it was possible to localize learning to an identifiable set of synapses in the mammalian brain.

memory. Attempts to treat human neurological diseases have led to the localization of areas of the brain involved in the formation and recall of memories. Epilepsy is a disorder characterized by uncontrollable increases in neural activity in specific parts of the brain. The resulting seizures, or "epileptic fits," can endanger the afflicted individual. In the past, serious cases of epilepsy were sometimes treated by destroying the part of the brain from which the surge of activity originated.

To find the right area, the surgery was done under local anesthesia, and different regions of the brain were electrically stimulated with fine electrodes while the patient reported on the resulting sensations. When some regions of association cortex were stimulated, patients reported vivid

46.15 The Conditioned Eye-Blink Reflex Depends on a Cerebellar Circuit

A small and specific area of the cerebellum is necessary for a rabbit to form a conditioned reflex.

EXPERIMENT

Question: Where is a conditioned reflex stored in the brain?

METHOD

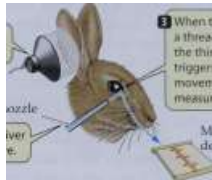
f

A speaker delivers a sound to the rabbit.

Speaker

T

A tube is used to deliver a puff of air to the eye.



Air nozzle

§J When the eye blinks, a thread attached to the third eyelid triggers the eye movement measuring device.

Measuring device

Experiment 1 Training the conditioned response

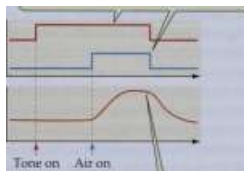
RESULTS

Tone ° n Off

Air puff Qff

Eye blink

Paired stimuli are delivered to the animal: first the tone, then the air puff.



T 1

Tone on Air on

The rabbit blinks in response to the air puff. Before training, the tone alone did not elicit an eye-blink response.

Experiment 2 Testing the conditioned response RESULTS

When the tone alone is delivered after several days of training, the rabbit blinks its eye.

Tone

On

Off Air puff Qff

Eye blink

Experiment 3

RESULTS

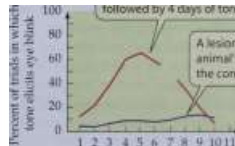


i

Tone on

Destroy a small part of the rabbit's cerebellum and test the effect of the lesion on the conditioned reflex.

A non-lesioned animal shows conditioned reflex during 5 days of paired stimuli followed by 4 days of tone delivery only.



A lesion results in the animal's inability to learn the conditioned reflex.

3 4 5 6 7 Time (days)

9 10 11

Conclusion: The cerebellar nucleus is necessary for learning this conditioned reflex.

828 CHAPTER FORTY-SIX

memories. Such observations were the first evidence that

memories have anatomical locations in the brain and exist, b properties of neurons and networks of neurons. Yet the destruction of a small area does not completely erase a memory so it is postulated that memory is a function distributed over many brain regions and that a memory may be stimulated via many different routes.

You can recognize several forms of memory from your own experience. There is immediate memory for events that are happening now. Immediate memory is almost perfectly photographic, but it lasts only seconds. Short-term memory contains less information, but it lasts longer—on the order of 10 to 13 minutes. If you are introduced to a group of new people, you may remember most of their names for 5 or 10 minutes, but you will have forgotten them in an hour or so if you have not repeated them, written them down, or used them in a conversation. Repetition, use, or reinforcement by something that gets your attention (such as the title President) facilitates the transfer of short-term memory to long-term memory, which can last for days, months, or years.

Knowledge about neural mechanisms for the transfer of short-term memory to long-term memory has come from observations of persons who have lost parts of the limbic system, notably the hippocampus. A famous case is that of a man identified as H.M., whose hippocampus on both sides of the brain was removed in an effort to control severe epilepsy. Since that surgery, H.M. has not been able to transfer information to long-term memory. If someone is introduced to him, has a conversation with him, and then leaves the room, when that person returns he or she is unknown to H.M.—it is as if the previous conversation had never taken place. H.M. retains memories of events that happened before his surgery, but he remembers post-surgery events for only 10 or 15 minutes.

Memory of people, places, events, and things is called declarative memory because you can consciously recall and describe them. Another type of memory, called procedural memory, cannot be consciously recalled and described: It is the memory of how to perform a motor task. When you learn to ride a bicycle, ski, or use a computer keyboard, you form procedural memories. Although H.M. is incapable of forming declarative memories, he is capable of forming procedural memories. When taught a motor task day after day, he cannot recall the lessons of the previous day, yet his performance steadily improves. Thus procedural learning and memory must involve mechanisms different from those used in declarative learning and memory.

Our understanding of learning and memory in cellular terms is very rudimentary. New techniques that enable functional imaging of the brain in ways that reveal changes in the metabolic activity of specific regions and structures are greatly enhancing progress in this area.

Language abilities are localized in the left cerebral hemisphere

No aspect of brain function is as integrally related to human consciousness and intellect as is language. There-

fore, studies of the brain mechanisms that underlie the acquisition and use of language are extremely interesting to neuroscientists. A curious observation about language abilities is that they are usually located in only one cerebral hemisphere—which in 97 percent of people is the left hemisphere. This phenomenon is referred to as the lateralization of language functions.

Some of the most fascinating research on this subject was conducted by Roger Sperry and his colleagues at the California Institute of Technology; Sperry received the Nobel prize in medicine for this work. The two cerebral hemispheres are connected by a tract of white matter called the corpus callosum. In one severe form of epilepsy, bursts of action potentials travel from hemisphere to hemisphere across the corpus callosum. Cutting the tract eliminates the problem, and patients function nearly normally following the surgery. But experiments revealed interesting deficits in the language abilities of these "split-brain" persons. Without the connection between the two hemispheres, the knowledge or experience of the right hemisphere could no longer be expressed in language, nor could language be used to communicate with the right hemisphere.

Another curious feature of our nervous systems is that the left side of the body is served (in both sensory and motor aspects) mostly by the right side of the brain, and the right side of the body is served mostly by the left side of the brain. Thus, sensory input from the right hand goes to the left cerebral hemisphere, and sensory input from the left hand goes to the right

cerebral hemisphere. Language abilities reside predominantly in the left hemisphere.

The mechanisms of language in the left hemisphere have been the focus of much research. Again, the experimental subjects are persons who have suffered damage to the left hemisphere and are left with one of many forms of aphasia, a deficit in the ability to use or understand words. These studies have identified several language areas in the left hemisphere (Figure 46.16).

Broca's area, located in the frontal lobe just in front of the motor cortex, is essential for speech. Damage to Broca's area results in halting, slow, poorly articulated speech or even complete loss of speech, but the patient can still read and understand language. In the temporal lobe, close to its border with the occipital lobe, is Wernicke's area, which is more involved with sensory than with motor aspects of language. Damage to Wernicke's area can cause a person to lose the ability to speak sensibly while retaining the abilities to form the sounds of normal speech and to imitate its cadence. Moreover, such a patient cannot understand spoken or written language. Near Wernicke's area is the angular gyrus, which is believed to be essential for integrating spoken and written language.

Normal language ability depends on the flow of information among various areas of the left cerebral cortex. Input from spoken language travels from the primary auditory cortex to Wernicke's area (see Figure A6A6a). Input from written language travels from the primary visual cortex to the angular gyrus to Wernicke's area (see Figure



46.76 Language Areas of the Cortex

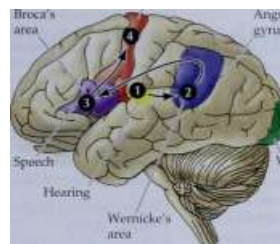
Different regions of the left cerebral cortex participate in the processes of (a) repeating a word that is heard versus (b) repeating a written word.

(a) Repeating a heard word

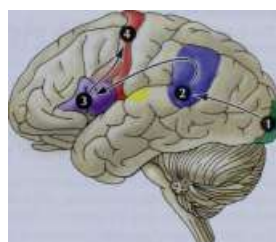
Motor

(b) Speaking a written word

Angular gyrus



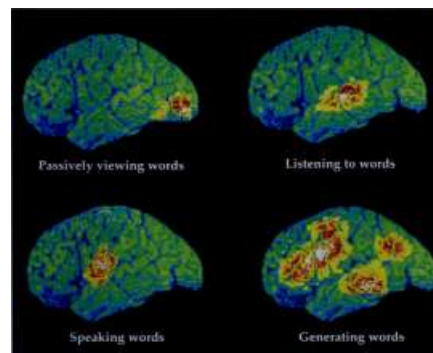
Vision



46.16fr). Commands to speak are formulated in Wernicke's area and travel to Broca's area and from there to the primary motor cortex. Damage to any one of those areas or the pathways between them can result in aphasia. Using modern methods of functional brain imaging, it is possible to see the metabolic activity in different brain areas when the brain is using language (Figure 46.17).

What is consciousness?

This chapter has only scratched the surface of our knowledge about the organization and functions of the human brain, but it may give you some idea of the incredible challenge that neurobiologists face in trying to understand their own brains. Progress is being aided by powerful new technologies, such as patch clamping (see Chapter 44), functional imaging (see Figure 46.17), and neurochemical and molecular methods. However, even these sophisticated new research tools may not allow us to answer the question "What is consciousness?"



If you look at a black dog, you are conscious of the fact that it is a dog, it is black, and it is a Labrador retriever, and you may remember that its name is Sarina, it is 3 years old, it belongs to your friend Meera, and so on. From what you have learned in this chapter, imagine how many neurons would be active during this experience: neurons in the visual system, the language areas, and in different regions of association cortex. But is being conscious of the black dog simply a result of the fact that all of these neurons are firing at the same time? Your brain is simultaneously processing many other sensory inputs, but you are not necessarily conscious of those inputs. What makes you conscious of the black dog and associated memories and not conscious of other information the brain is processing at the same time? If we could describe all the neurons and all the synapses involved in the conscious experience of seeing and naming a black dog, and then build a computer with devices that modeled all these neurons and connections, would that computer be conscious? It has been said that the question of consciousness resolves into two types of problems: "easy" and "hard." The easy problems deal with all the cells and circuits that process the information that is involved in conscious experience. The implication of "easy" is that we seem to have the tools to solve these kinds of problems, as complex as they may be. The hard problems involve explaining how properties of cells and networks result in consciousness, and we seem to lack the proper tools or concepts even to begin to solve these problems.

46.17 Imaging Techniques Reveal Active Parts of the Brain

Positron emission tomography (PET) scanning reveals the brain regions that are activated by different aspects of language use. A radioactive form of glucose is given to the subject. Radioactivity accumulates in brain areas in proportion to their metabolic use of glucose. The PET scan visualizes levels of radioactivity in specific brain regions.

830 CHAPTER FORTY-SIX

Chapter Summary

The Nervous System: Structure, Function, and Information Flow

- The brain and spinal cord make up the central nervous

-\ -torn: the cranial and spinal nerves make up the peripheral nei \ ous Sj -tern. A nerve is a bundle of many axons carrying information to and from the central nervous system. Review Figure 46.1

- The nervous system can be modeled conceptually in terms of the direction of information flow and whether or not we are conscious of the information. Review Figure 46.2
- The vertebrate nervous system develops from a hollow dorsal neural tube. The brain forms from three swellings at the anterior end of this neural tube, which become the hind-brain, the midbrain, and the forebrain. Review Figure 46.3
- The forebrain develops into the cerebral hemispheres (the telencephalon) and the underlying thalamus and hypothalamus (the diencephalon). The midbrain and hindbrain develop into the brain stem. More primitive and autonomic functions are localized in the brain stem, and conscious experience depends on the cerebrum.

Functional Subsystems of the Nervous System

- The nervous system is composed of many subsystems that function simultaneously. Some important subsystems are the spinal cord, the reticular system, the limbic system, and the cerebrum.
- The spinal cord communicates information between the brain and the body. It also processes and integrates much information, and can issue some commands to the body without input from the brain. Review Figure 46.4
- The reticular system of the brain stem is a complex network that directs incoming information to appropriate brain stem nuclei that control autonomic functions, as well as transmitting the information to the forebrain that results in conscious sensation. The reticular system controls the level of arousal of the nervous system. Review Figure 46.5
- The limbic system is an evolutionarily primitive part of the forebrain that is involved in emotions, physiological drives, instincts, and memory. Review Figure 46.6
- The cerebral hemispheres are the dominant structures of the human brain. Their surfaces consist of a layer of neurons called the cerebral cortex.
- Most of the cerebral cortex is involved in higher-order information processing, and these areas are generally called

association cortex.

► The cerebral hemispheres can be divided into temporal, frontal, parietal, and occipital lobes. Many motor functions are localized in parts of the frontal lobe, information from many receptors around the body projects to a region of the parietal lobe, visual information projects to the occipital lobe, and auditory information projects to a region of the temporal lobe. Review Figures 46.7, 46.8, 46.9, 46.10

Information Processing by Neuronal Circuits

► The functions of the nervous system are beginning to be understood in terms of the properties of cells organized in neuronal circuits.

► The autonomic nervous system consists of efferent pathways that control the organs and organ systems of the body. Its sympathetic and parasympathetic divisions normally work in opposition to each other. These divisions are characterized by their anatomy, neurotransmitters, and effects on target tissues. Review Figure 46.11

► Neuronal circuits in the occipital cortex integrate visual information. Receptive field responses of retinal ganglion

cells are communicated to the brain in the optic nerves. This information is projected to the visual cortex in such a way as to create receptive fields for cortical cells.

► A simple cell is stimulated by a bar of light with a specific orientation falling at a specific location on the retina. A complex cell is maximally stimulated by such a stimulus moving across the retina. The visual cortex seems to assemble a mental image of the visual world by analyzing edges of patterns of light. Review Figure 46.12

► Binocular vision results from circuits that communicate information from both eyes to binocular cells in the visual cortex. These cells interpret distance by measuring the disparity between where the same stimulus falls on the two retinas. Review Figure 46.13

Understanding Higher Brain Functions in Cellular Terms

► Humans have a daily cycle of sleep and waking. Sleep can be divided into slow-wave (non-REM) sleep and rapid-eye-movement (REM) sleep. Human non-REM sleep is divided into four stages of increasing depth. Review Figure 46.14

► Some learning and memory processes have been localized to specific brain areas. Repeated activation of identified circuits in brain regions such as the hippocampus have revealed long-lasting changes in synaptic properties referred to as long-term potentiation and long-term depression, which may be involved in learning and memory. Review Figure 46.15

Complex memories can be elicited by stimulating small regions of association cortex. Damage to the hippocampus can destroy the ability to form long-term declarative memories, but not procedural memories.

► Language abilities are localized mostly in the left cerebral hemisphere, a phenomenon known as lateralization.

► Different areas of the left hemisphere—including Broca's area, Wernicke's area, and the angular gyrus—are responsible for different aspects of language. Review Figure 46.16

For Discussion

1. The mammalian nervous system begins as a hollow tubular structure, but just before the three regions of the brain begin to form, this hollow tube constricts at the site that will become the junction between the brain and the spinal cord. What could be the significance of this constriction? What does it suggest about the mechanism by which the brain develops?
2. The stretch receptors in muscles are modified muscle fibers, and they have their own motor neurons. What is the function of these motor neurons? To think about this question, remember that the function of the monosynaptic reflex is to adjust muscle tension to a change in load so that the position of the limb does not change.
3. A patient is unable to speak coherently. He can read and write, and he has no obvious loss of muscle function. Where would you expect to find an abnormality if you did brain scans of this patient?
4. We described the organization of the visual cortex as columns of cells that alternately receive input from the left eye and the right eye. If a young kitten is allowed to see light out of only one eye for a day, more synapses begin to form in the cortical columns receiving input from that eye, while synapses decrease in the intervening columns. This redistribution of synapses does not occur, however, if the kitten is not allowed to sleep. What hypotheses could you propose on the basis of these results?

Effectors: Making Animals Move



The central nervous system is more v^{\wedge} than a processor and storage medium for $^{\wedge}$ information. It also allows animals to respond to that information. Effectors are tissues and organs commanded by the CNS to carry out these responses, and most responses of animals involve movement.

A fascinating array of adaptations enable animals to move. Consider the act of jumping. When you jump, neural signals from the visual cortex of your brain are routed through spinal circuits that tell certain leg muscles to contract, extending your legs, and hence you jump. Highly skilled and trained athletes can actually outleap their own body height.

But as "record jumpers" go, many other animals—cats, spiders, kangaroos, and fleas to name just a few—far surpass even the Olympian feats of humans. A flea, for example, can jump more than 200 times its body length. Unlike a human jumper, the flea's jumping mechanism doesn't involve muscles but works like a slingshot. The flea is so small, and its initial acceleration is so great, that no muscle can contract fast enough to cause such a movement. Instead, at the base of its jumping legs is an elastic material that is compressed by muscles while the flea is resting. When a trigger mechanism is released, the elastic material recoils and "fires" the flea up and over to its target (or away from an enemy).

Jumping is just one adaptation an animal can use to respond to information received by its sensory receptors. Effectors include the internal organs and organ systems that the animal uses to control its internal environment; these effectors are the subjects of subsequent chapters. In this chapter, we focus on cilia, flagella, muscles, and skeletons—the mechanisms that create mechanical forces and use those forces to change shape and move, and which are the basis for most animal behavior. At the end we will briefly consider a few effectors other than those that create movement.

A Champion Jumps

Sergey Kliugin of Russia won the gold medal for high jumping in the 2000 Summer Olympics. His winning jump was 2.35 meters, or about 1.3 times his height.

Cilia, Flagella, and Cell Movement

Two subcellular structures, microtubules and microfilaments (see Figure 4.21), generate cell movement. Both of these structures consist of long protein molecules that can change length or shape. Microtubules generate the small-scale movements of cilia and flagella. Microfilaments reach their highest level of organization in muscle cells, which generate large-scale movements.

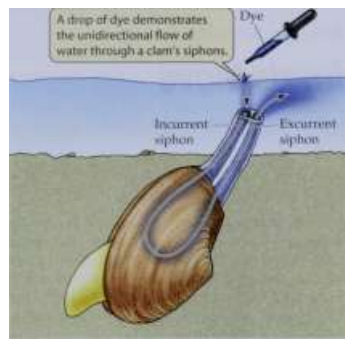
Cilia are tiny, hairlike appendages of cells

Certain protists are covered by dense patches of cilia that propel them through their aqueous environment. Each cilium is tiny, about 0.25 μm in diameter. Multicellular animals use ciliated cells to move liquids and particles over cell surfaces. Many invertebrates use ciliated cells to obtain food and oxygen. Some mollusks, for example, use cilia to circulate a current of water across their gas exchange and feeding surfaces (Figure 47.1).

The airways of many animals are lined with and cleaned by ciliated cells (Figure 47.2). In humans, the cilia continuously sweep a layer of mucus from deep down in the lungs, up through the windpipe, and into the throat. The mucus carries particles of dirt and dead cells. We can then either swallow or spit out the mucus, and with it the trapped detritus. Ciliated cells lining the female reproductive tract create currents that sweep eggs from the ovaries into the oviducts and all the way down to the uterus.



A drop of dye demonstrates the unidirectional flow of water through a clam's siphons.



47.1 Cilia Create Water Currents in a Clam's Siphons

In burrowing mollusks such as clams, cilia lining the siphons maintain a unidirectional flow of water: in one siphon, over the gills, and out the other siphon. The gills extract oxygen and food from this flow of water.

A cilium moves with the same basic motion as a swimmer's arms during the breaststroke (Figure 47.3a). During the power stroke, the cilium projects stiffly outward and moves backward, propelling the cell forward (or the medium backward). During the recovery stroke, the cilium folds as it returns to its original position. The power stroke is fast, the recovery stroke slow. As you know from moving your arms or legs in water, there is less resistance the slower you move. The resistance of the medium to the recovery stroke is thus slight compared with its resistance to the power stroke. Groups of cilia typically beat in coordinated waves. At any particular moment, some cilia of a cell are moving through the power stroke and others are recovering.

Flagella are like long cilia

The flagella of eukaryotes are identical to cilia except that they are longer and occur singly, or in groups of only a few, on any one cell. Flagellated cells maintain a flow of water through the bodies of sponges, bringing in food and oxygen and removing carbon dioxide and wastes. Flagella power the movement of the sperm of most species. Because of their greater length, flagella have a whiplike stroke pattern rather than the "swimming" stroke pattern of cilia (Figure 47.3b).

Cilia and flagella are moved by microtubules

The core of a cilium or a flagellum is called the axoneme. The axoneme contains a ring of nine pairs of microtubules. In the center of the ring may be an additional pair of microtubules, a single microtubule, or no microtubule. As dis-

(«)



47.2 Cilia Line Respiratory Passages

A scanning electron micrograph of a rabbit's airway shows many cilia.

As discussed in Chapter 4, microtubules are hollow tubes formed from polymerization of the globular polypeptide tubulin. Other proteins in the axoneme form spokes, side arms, and cross-links. Side arms composed of the protein dynein generate force (see Figures 4.24 and 4.25). Dynein is an enzyme that catalyzes the hydrolysis of ATP and uses the released energy to change its shape, thereby generating mechanical force.

When the dynein arms on one microtubule pair contact a neighboring microtubule pair and bind to it, ATP is broken down, and the resulting conformational changes in the dynein molecules cause the arms to point toward the base of the axoneme (Figure 47.4). This action pushes the microtubule pair ahead in relation to its neighbor. The dynein arms then detach from the neighboring pair and reorient to their starting horizontal position. As the cycle is repeated,

A cilium moves in a pattern similar to an arm of a swimmer doing the breaststroke.



Power stroke

Recovery stroke

(b)

Flagella are much longer than cilia. A flagellum moves in an undulating, whiplike pattern.

47.3 Cilia and Flagella Move Differently

Cilia (a) have a "swimmer's stroke," whereas flagella (6) have a whiplike motion.

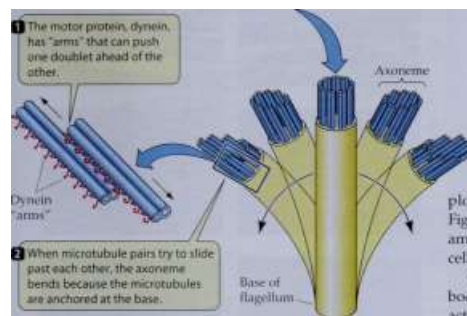
EFFECTORS: MAKING ANIMALS MOVE 833

Flagellum



SS ^

|The motor protein, dynein, has "arms" that can push one doublet ahead of the other.



Dynein

"arms"

| When microtubule pairs try to slide past each other, the axoneme bends because the microtubules are anchored at the base.

adjacent microtubule pairs try to "row" past each other, with the dynein arms acting as "oars." Axonemes severed from cells continue to flex in a normal pattern if exposed to calcium ions (Ca^{2+}) and ATP, demonstrating that the motile mechanism is part of the axoneme itself and not derived from the associated cell.

Microtubules are intracellular effectors

Microtubules, as components of the cytoskeleton, contribute to the shape of eukaryotic cells. Microtubules are important intracellular effectors for changing cell shape, moving organelles, and enabling cells to respond to their environment. Microtubules change the shapes of cells and move cells by polymerizing and depolymerizing the protein tubulin.

As we saw in Chapter 9, the spindle that moves chromosomes to the mitotic poles at anaphase is made up of microtubules. Another example of microtubule involvement in cell movement is the growth of the axons of neurons in the developing nervous system. Neurons find and make their appropriate connections by sending out long extensions that search for the correct contact cells. If polymerization of tubulin is chemically inhibited, the neurons do not extend.

J

Microfilaments change cell shape and cause cell movements

Microfilaments are proteins that change conformation as a means of generating forces. The dominant microfilament in animal cells is the protein actin. Bundles of cross-linked actin strands form important structural components of cells. The microvilli that increase the absorptive surface area of the cells lining the gut are stiffened by actin microfilaments (see Figure 4.23), as are the stereocilia of the sen-sow hair cells in the mammalian ear (see Figure 45.12). Actin microfilaments can change the shape of a cell simply by polymerizing and depolymerizing.

47.4 Microtubules Create Motion by Pushing Against Each Other

Cilia and flagella move because of interactions between microtubules in the axoneme.

Together with the protein myosin, actin microfilaments generate the contractile forces responsible for many aspects of cell movement and changes in cell shape. The contractile ring that divides an animal cell undergoing mitosis into two daughter cells is composed of actin microfilaments in association with myosin. The mechanisms that many cells employ to engulf materials (endocytosis; see Chapter 5 and ure 19.4) also rely on interactions between actin microfilaments and myosin. Nets of actin and myosin beneath the cell membrane change a cell's shape during endocytosis.

Certain cells in multicellular animals travel within the body by amoeboid movement, which is generated by the activity of actin microfilaments and myosin. During development, many cells migrate by amoeboid movement. Throughout an animal's

life, phagocytic cells circulate in the blood, squeeze through the walls of the blood vessels, and wander through the tissues by amoeboid movement. The mechanisms of amoeboid movement have been studied extensively in the protist for which this type of movement was named—the amoeba, which lives in freshwater streams and ponds (see the photograph on page 476).

Amoebas move by extending lobe-shaped projections called pseudopods and then seemingly squeezing themselves into those pseudopods. The cytoplasm in the core of the amoeba, called plasmasol, is relatively liquid, but just beneath the plasma membrane the cytoplasm is much thicker, and is called plasmagel. Reversible changes between plasmasol and plasmagel move the cytoplasm.

To form a pseudopod, the thick plasmagel in one area of the cell thins, allowing a bulge to form. Just under the cell surface, in the plasmagel, is a network of actin microfilaments that interacts with myosin to squeeze plasmasol into the bulge. As the microfilament network continues to contract, cytoplasm streams in the direction of the pseudopod. Eventually the cytoplasm at the leading edge of the pseudopod converts to plasmagel, and the pseudopod stops forming. Thus the basis for amoeboid motion is the ability of the cytoplasm to cycle through sol and gel states and the ability of the microfilament network under the cell membrane to contract and cause the cytoplasmic streaming that pushes out a pseudopod.

Muscle Contraction

Most behavioral and many physiological responses depend on muscle cells. Muscle cells are specialized for contraction and have high densities of actin and myosin. Such cells are found throughout the animal kingdom. Wherever whole tissues contract in animals, muscle cells are responsible.

In muscle cells, actin and myosin molecules are organized into filaments consisting of two or more molecules.

834 CHAPTER FORTY-SEVEN

Muscle fiber Intercalated discs

47.5 Vertebrate Muscle Tissue

The fibers of cardiac, or heart muscle (top), branch and create a meshwork that resists tearing or breaking. Intercalated discs provide strong mechanical adhesions between the cells. In smooth muscle (center), the cells are usually arranged in sheets. Skeletal muscle (bottom) appears striped, or striated. The individual cells, called muscle fibers, are very large and are multinucleated.

Actin filaments consist of two actin molecules twisting around each other, and myosin filaments are bundles of many myosin molecules. The actin and myosin filaments lie parallel to each other. When contraction is triggered, the actin and myosin filaments slide past each other in a telescoping fashion.

There are three types of vertebrate muscle: smooth muscle, cardiac (heart) muscle, and skeletal muscle

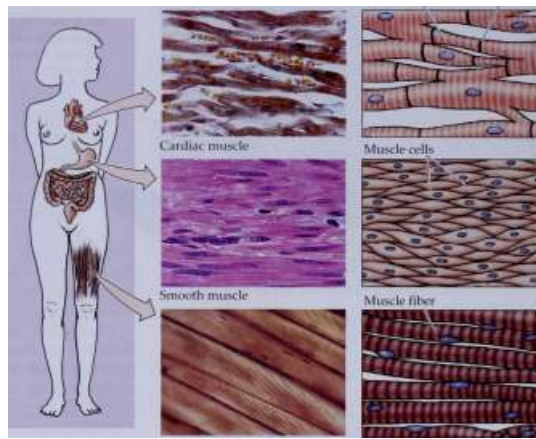
(Figure 47.5). Contraction in all three types is triggered by action potentials moving along their membranes. Although they all use the same contractile mechanism, these three muscle types have important differences that adapt them to their particular functions.

Smooth muscle causes slow contractions of many internal organs

Smooth muscle provides the contractile force for most of our internal organs, which are under the control of the autonomic nervous system. Smooth muscle moves food through the digestive tract, controls the flow of blood through blood vessels, and empties the urinary bladder. Structurally, smooth muscle cells are the simplest muscle cells. They are usually long and spindle-shaped, and each cell has a single nucleus. Because the filaments of actin and myosin in smooth muscle are not as regularly arranged as those in the other muscle types, the contractile machinery is not obvious when the cells are viewed under the light microscope (Figure 47.5, center).

If we study smooth muscle tissue from a particular organ, such as the wall of the digestive tract, we find that it has some interesting properties. The cells are arranged in sheets, and individual cells in the sheets are in electrical contact with one another through gap junctions. As a result, an action potential generated in the membrane of one smooth muscle cell can spread to all the cells in the sheet of tissue.

Another interesting property of a smooth muscle cell is that the resting potential of its membrane is sensitive to being stretched. If the wall of the digestive tract is stretched in one location (as by receiving a mouthful of food), the membranes of the stretched cells depolarize, reach thresh-



Skeletal muscle

old, and fire action potentials that cause the cells to contract. Thus smooth muscle contracts after being stretched, and the harder it is stretched, the stronger the contraction. (Later in this chapter we will see how membrane depolarization triggers contraction.)

Other factors that alter the membrane potential of smooth muscle cells are the neurotransmitters of the autonomic nervous system (see Figure 46.11). In the case of the digestive tract, acetylcholine causes smooth muscle cells to depolarize and thus makes them more likely to fire action potentials and contract. Norepinephrine causes these muscle cells to hyperpolarize and therefore makes them less likely to fire action potentials and contract (Figure 47.6).

Cardiac muscle causes the heart to beat

Cardiac muscle looks different from smooth muscle or skeletal muscle when viewed under the microscope (Figure 47.5, top). The cells appear striped, or striated, because of the regular arrangement of bundles of actin and myosin filaments within them. Actin and myosin are arranged in a similar way in skeletal muscle, as we'll see below.

The unique feature of cardiac muscle cells is that they branch. The branches of adjoining cells are interdigitated into a meshwork that gives cardiac muscle an ability to resist tearing. As a result, the heart walls can withstand high pressures while pumping blood without the danger of developing leaks. Also adding to the strength of cardiac muscle are intercalated discs that provide strong mechanical adhesions between adjacent cells.

As is true of smooth muscle, the individual cells in a sheet of cardiac muscle are in electrical contact with one another. Gap junctions present in the intercalated discs present low resistance to ions or electric currents. Therefore, a

EXPERIMENT

Question: What stimulates contraction of smooth muscle?

METHOD Incubate a strip of smooth (intestinal) muscle in a saline bath. Measure action potentials and force of contraction. Experiment 1 Stretch intestinal muscle and analyze response.

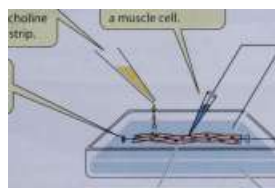
Q In experiment #1, the muscle strip is stretched, in experiment #2 a pipette drips acetylcholine or norepinephrine onto strip.

QThe muscle is anchored to a device that applies stretch.

§J An electrode detects action potentials in a muscle cell.

f

Muscle membrane potential and action potentials are recorded.



/

Measuring electrode

T

Chart recorder

Amplifier

L

jpimiJLum

Reference electrode

-rmra—

Force transducer

Intestinal muscle

' Saline bath

Measures

muscle contractions

RESULTS

Stretching depolarizes the smooth muscle membrane. The depolarization causes action potentials that activate the contractile mechanism.

t

Chart recorder

The force of contraction of the muscle is measured.

Experiment 2 Response of muscle strip to neurotransmitters of the autonomic nervous system.

When acetylcholine is dripped onto the muscle, the cells depolarize, fire action potentials more rapidly, and increase their force of contraction.

Apply acetylcholine

I

Wash out acetylcholine

F

r n

Norepinephrine, on the other hand, causes the cells to hyperpolarize, decrease their rate of firing, and decrease their force of contraction.

Apply 1 Wash out

norepinephrine I norepinephrine

+25

0

Membrane potential (mV) -25

-50

Force

^AAAAA

y —\.

r Muscle ! ^-

contracts

Muscle r^v relaxes

*AAXkA*kJ

i/~

RESULTS

Autonomic neurotransmitters alter membrane resting potential and rate that smooth muscle cells fire action potentials.

Conclusion: Smooth muscle contraction is stimulated by stretch and by the parasympathetic neurotransmitter acetylcholine.

47.6 Smooth Muscle Action

Stretching depolarizes the membrane of smooth muscle cells, and this depolarization causes action potentials that activate the contractile mechanism. The neurotransmitters acetylcholine and norepinephrine also alter the membrane potential of smooth muscle, making it more or less likely to contract.

depolarization initiated at one point in the heart spreads rapidly through the mass of cardiac muscle.

An interesting feature of vertebrate cardiac muscle is that certain specialized muscle cells, called pacemaker cells, initiate the rhythmic contractions of the heart. We'll learn about the molecular basis for this pacemaking func-

tion in Chapter 49. Because of these specialized pacemaker cells, the heartbeat is myogenic —generated by the heart muscle itself. The autonomic nervous system modifies the rate of the pacemaker cells, but is not essential for their continued rhythmic function. A heart removed from an animal continues to beat with no input from the nervous system. The myogenic nature of the heartbeat is a major factor in making heart transplants possible.

Skeletal muscle carries out behavior

Skeletal muscle carries out, or effects, all voluntary movements, such as running or playing a piano, and generates the movements of breathing. Skeletal muscle is also called

836 CHAPTER FORTY-SEVEN

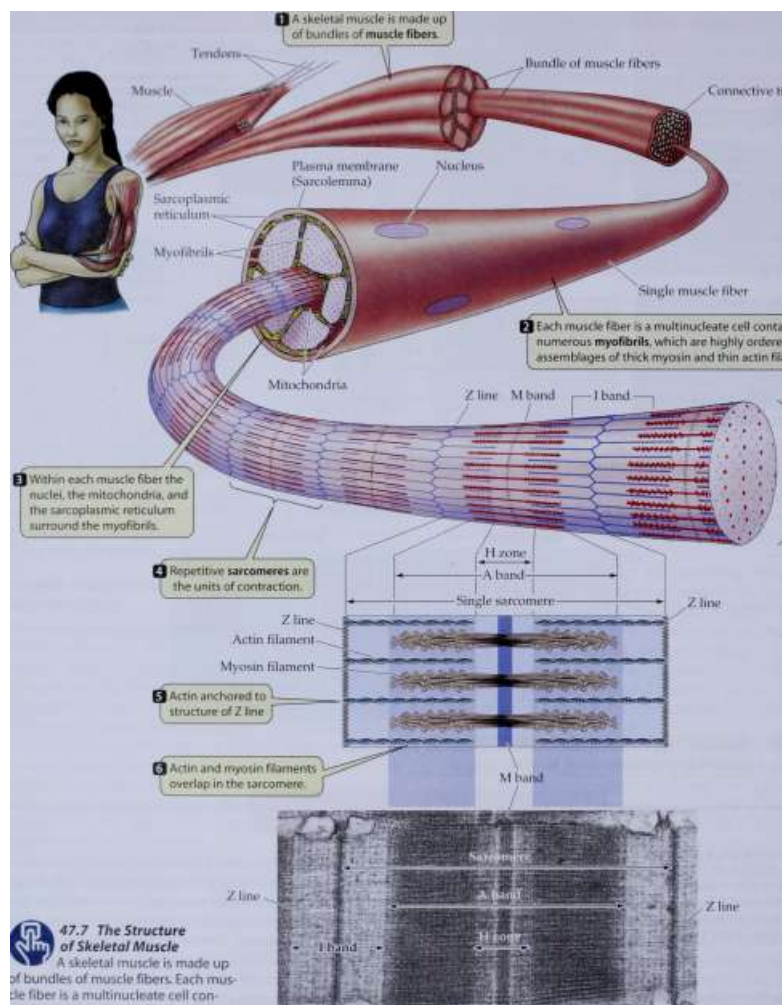
{

A skeletal muscle is made up of bundles of muscle fibers.

Bundle of muscle fibers

Connective tissue

! Each muscle fiber is a multinucleate cell containing numerous myofibrils, which are highly ordered assemblages of thick myosin and thin actin filaments.



> Single myofibril

Z line



47.7 The Structure of Skeletal Muscle

A skeletal muscle is made up of bundles of muscle fibers. Each muscle fiber is a multinucleate cell containing numerous myofibrils, which are highly ordered assemblages of thick myosin and thin actin filaments. The structure of the myofibrils gives muscle fibers their characteristic striated (striped) appearance.

Q The structure of the myofibrils gives muscle fibers their characteristic striated appearance, as seen in this electron micrograph. Where there are only actin filaments the myofibril appears light; where there are both actin and myosin filaments the myofibril appears dark.

striated muscle because the highly regular arrangement of its actin and myosin filaments gives it a striped appearance. Skeletal muscle cells, called muscle fibers, are large. Unlike smooth muscle and cardiac muscle cells, each of which has a single nucleus, skeletal muscle fibers have many nuclei because they develop through the fusion of many individual cells. A muscle such as your biceps (which bends your arm) is composed of many muscle fibers bundled together by connective tissue.

What is the relation between a muscle fiber and the actin and myosin filaments responsible for its contraction? Each muscle fiber is composed of myofibrils —bundles of contractile filaments made up of actin and myosin (Figure 47.7). Within each myofibril are thin actin filaments and thick myosin filaments. If we cut across the myofibril at certain locations, we see only thick filaments; if we cut at other locations, we see only thin filaments. But, in most regions of the myofibril, each thick myosin filament is surrounded by six thin actin filaments.

A longitudinal view of a myofibril reveals the reason for the striated appearance of skeletal muscle. The band pattern of the myofibril is due to repeating units called sarcomeres, which are the units of contraction (see Figure 47.7). Each sarcomere is made of overlapping filaments of actin and myosin. As the muscle contracts, the sarcomeres shorten, and the appearance of the band pattern changes.

The observation that the widths of the bands in the sarcomeres change when a muscle contracts led two British biologists, Hugh Huxley and Andrew Huxley, to propose a molecular mechanism of muscle contraction. Let's look at the band pattern of the myofibril in detail (see the micrograph in Figure 47.7). Each sarcomere is bounded by Z lines, which are structures that anchor the thin actin filaments. Centered in the sarcomere is the A band, which contains all the myosin filaments. The H zone and the I band, which appear light, are regions where actin and myosin filaments do not overlap in the relaxed muscle. The

dark stripe within the H zone is called the M band; it contains proteins that help hold the myosin filaments in their regular hexagonal arrangement.

When the muscle contracts, the sarcomere shortens. The H zone and the I band become much narrower, and the Z lines move toward the A band as if the actin filaments were sliding into the region occupied by the myosin filaments. This observation led Huxley and Huxley to propose the sliding filament theory of muscle contraction: Actin and myosin filaments slide past each other as the muscle contracts.

To understand what makes the filaments slide, we must examine the structure of actin and of myosin (Figure 47.8). Each myosin molecule consists of two long polypeptide chains coiled together, each ending in a large globular head. A myosin filament is made up of many

myosin molecules arranged in parallel, with their heads projecting laterally from one or the other end of the filament. The actin filament consists of a helical arrangement of two chains of monomers twisted together like two strands of pearls. Twisting around the actin chains is another protein, tropomyosin, and attached to it at intervals are molecules of troponin. We'll discuss the roles of these last two proteins in the following section.

The myosin heads have sites that can bind to actin and thereby form bridges between the myosin and the actin filaments. The myosin heads also have ATPase activity; that is, they bind and hydrolyze ATP. The energy released when this happens changes the orientation of the myosin head.

Together, these details explain the cycle of events that cause the actin and myosin filaments to slide past each other and shorten the sarcomere. A myosin head binds to an actin filament (see Figure 47.8). Upon binding, the head changes its orientation with respect to the myosin filament, thus exerting a force that causes the actin and myosin filaments to slide about 5 to 10 nm relative to each other. Next, the myosin head binds a molecule of ATP, which causes it to release the actin. When the ATP is hydrolyzed, the energy released causes the myosin head to return to its original conformation, in which it can bind again to actin. It is as if the energy from ATP hydrolysis is being used to cock the hammer of a pistol, and contact of the myosin head with an actin binding site pulls the trigger.

We have been discussing the cycle of contraction in terms of a single myosin head. Don't forget that each myosin filament has many myosin heads at both ends and is surrounded by six actin filaments; thus the contraction of the sarcomere involves a great many cycles of interaction between actin and myosin molecules. That is why when a single myosin head breaks its contact with actin, the actin filaments do not slip backward.

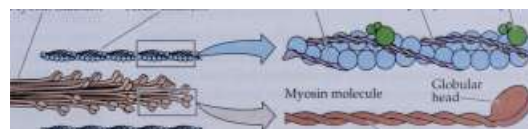
An interesting aspect of this contractile mechanism is that ATP is needed to break the actin-myosin bonds, but not to form them. Thus muscles require ATP to stop con-

Troponin has three subunits: one binds actin, one binds tropomyosin, and one binds Ca^{2+} .

Myosin filament Actin filament

Actin monomers Tropomyosin

Troponin



Linear polypeptide chain

47.8 Actin and Myosin Filaments Overlap to Form Myofibrils

Myosin filaments are bundles of molecules with globular heads and polypeptide tails. Actin filaments consist of two chains of actin monomers twisted together. They are wrapped by chains of the polypeptide tropomyosin and studded at intervals with another protein, troponin.

838 CHAPTER FORTY-SEVEN

Motor neuron

bracting. This fact explains why muscles

stiffen soon after animals die, a condition known as rigor mortis. Death stops the replenishment of the ATP stores of muscle cells, so the actin-myosin bonds cannot be broken, and the muscles stiffen. Eventually the proteins begin to lose their integrity, and the muscles soften. These events have regular time courses that differ somewhat for different regions of the body; therefore, an examination of the stiffness of the muscles of a corpse sometimes can help a coroner estimate the time of death.

Actin-myosin interactions are controlled by Ca^{2+}

Muscle contractions are initiated by action potentials from motor neurons arriving at the neuromuscular junction (see Figure 44.16). Motor neurons are generally highly branched and can synapse with up to a hundred muscle fibers each. All the fibers

activated by a single motor neuron constitute a motor unit and contract simultaneously in response to the action potentials fired by that motor neuron. To understand the fine control the nervous system has over muscle contraction, we must examine the membrane system of the muscle fiber and some additional protein components of the actin filaments.

Like neurons, vertebrate skeletal muscle fibers are excitable cells: When they are depolarized to a threshold that opens their voltage-gated sodium channels, their plasma membranes generate action potentials, just as the membranes of axons do. When an action potential arrives at the neuromuscular junction, neurotransmitter from the motor neuron binds to receptors in the postsynaptic membrane, causing ion channels in the motor end plate to open. Most of the ions that flow through these channels are K^+ , and therefore the motor end plate is depolarized.

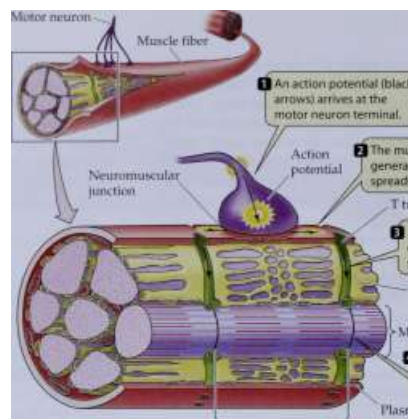
The depolarization of the motor end plate spreads to the surrounding plasma membrane of the muscle fiber, which contains voltage-gated ion channels. When threshold is reached, the plasma membrane fires an action potential that is conducted rapidly to all points on the surface of the muscle fiber.

The action potential in a muscle fiber also travels deep within the cell. The plasma membrane of the muscle fiber is continuous with a system of tubules that descends into and branches throughout the cytoplasm (also called the sar-coplasm) of the muscle fiber (Figure 47.9). The action potential that spreads over the plasma membrane of the muscle fiber also spreads through this system of transverse tubules, or T tubules.

The T tubules come into very close contact with a network of intracellular membranes called the sarcoplasmic reticulum that extends throughout the sarcoplasm, surrounding every myofibril. Calcium pumps in the sarcoplas-

Q An action potential (black arrows) arrives at the motor neuron terminal.

T tubule



The muscle fiber plasma membrane generates an action potential that spreads down T tubules...

...which causes the release of Ca^{2+} stored in the sarcoplasmic reticulum.

Sarcoplasmic reticulum

Myofibril

Released Ca^{2+} stimulates muscle contraction.

Sarcomere

Plasma membrane

ra



47.9 T Tubules in Action

An action potential at the neuromuscular junction spreads throughout the muscle fiber via a network of T tubules, triggering the release of Ca^{2+} from the sarcoplasmic reticulum.

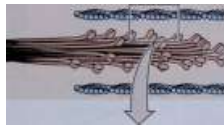
mic reticulum cause this membrane-enclosed compartment of the cell to take up Ca^{2+} ions from the sarcoplasm. Therefore, when the muscle fiber is at rest, there is a high concentration of Ca^{2+} in the sarcoplasmic reticulum and a low concentration of Ca^{2+} in the sarcoplasm surrounding the myofibrils.

When an action potential spreads through the T tubule system, it causes calcium channels in the sarcoplasmic reticulum to open, resulting in the diffusion of Ca^{2+} ions out of the sarcoplasmic reticulum and into the sarcoplasm surrounding the myofibrils. The Ca^{2+} stimulates the interaction of actin and myosin and the sliding of the filaments. How does this work?

An actin filament, as we have seen, is a helical arrangement of two strands of actin monomers. Lying in the grooves between the two actin strands is the two-stranded protein tropomyosin (see Figure 47.8). At regular intervals, the filament also

includes another globular protein, troponin. The troponin molecule has three subunits: one binds actin, one binds tropomyosin, and one binds Ca^{2+} .

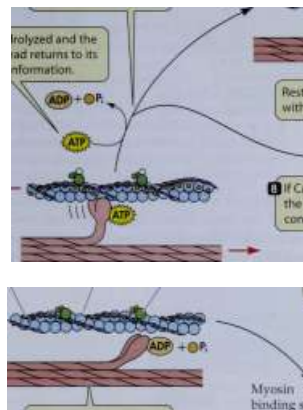
When Ca^{2+} is sequestered in the sarcoplasmic reticulum, the tropomyosin strands block the sites on the actin filament where myosin heads can bind. When the T tubule system depolarizes, Ca^{2+} is released into the sarcoplasm, where it binds to the troponin, changing the shape of the troponin molecule. Because the troponin is bound to the tropomyosin, this conformational change of the troponin twists the tropomyosin enough to expose the actin-myosin binding sites. Thus the cycle of making and breaking actin-myosin bonds is initiated, the filaments are pulled past one another, and the muscle fiber contracts. When the



| If Ca^{2+} is returned to the sarcoplasmic reticulum, the muscle relaxes.

Tropomyosin Actin filament Troponin

| ATP is hydrolyzed and the myosin head returns to its resting conformation.



\mr O An action potential arrives at the neuromuscular junction, causing a wave of depolarization in the T tubule system and the release of Ca^{2+} from the sarcoplasmic reticulum.

Q Ca^{2+} in the sarcoplasm binds troponin and exposes myosin-binding sites on the actin filament.

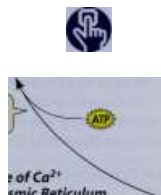
Resting myosin filament with ADP bound to head.

Myosin Ca^{2+} binding site

I If Ca^{2+} remains available, the cycle repeats and muscle contraction continues.

f

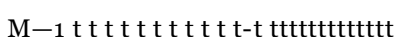
ATP binds to myosin, causing it to release actin.



k 47.10 The Release of Ca^{2+}

from the Sarcoplasmic Reticulum Triggers Muscle Contraction

When Ca^{2+} binds to troponin, it exposes actin-myosin binding sites. As long as binding sites and ATP are available, the cycle of actin and myosin interactions continues, and the filaments slide past each other.



Time

fiber increases and becomes more sustained. Thus an individual muscle fiber can show a graded response to increased levels of stimulation by its motor neuron.

At high levels of stimulation, the calcium pumps in the sarcoplasmic reticulum can no longer remove Ca^{2+} ions from the sarcoplasm between action potentials, and the contractile machinery generates maximum tension—a condition known as tetanus (Figure 47.11b). (Do not confuse this condition with the disease tetanus, which is caused by a bacterial toxin and is characterized by spastic contractions of skeletal muscles.)

How long a muscle fiber can maintain a tetanic contraction depends on its supply of ATP. Eventually the fiber will become fatigued. It may seem paradoxical that the lack of ATP causes fatigue, since the action of ATP is to break actin-myosin bonds. But remember that the energy released from the hydrolysis of ATP "recocks" the myosin heads, allowing them to cycle through another power stroke. When a muscle is contracting against a load, the cycle of making and breaking actin-myosin bonds must continue to prevent the load from stretching the muscle. The situation is like rowing a boat upstream: You cannot maintain your position relative to the stream bank by just holding the oars out against the current; you have to keep rowing. Likewise, actin-myosin bonds have to keep cycling to maintain tension in the muscle.

The ability of a whole muscle to generate different levels of tension depends on how many fibers in that muscle are activated. Whether a muscle contraction is strong or weak depends both on how many of the motor neurons that synapse with that muscle are firing and on the rate at which those neurons are firing. These two factors can be thought of as spatial summation and temporal summation, respectively.

Both types of summation increase the strength of contraction of the muscle as a whole. Faster twitching of individual fibers causes temporal summation (see Figure

47.11b), and an increase in the number of motor units involved in the contraction causes spatial summation. (Remember that a motor unit consists of all the muscle fibers innervated by a single neuron, and that a single muscle consists of many motor units.)

Many muscles of the body maintain a low level of tension called tonus even when the body is at rest. For example, the muscles of the neck, trunk, and limbs that maintain our posture against the pull of gravity are always working, even when we are standing or sitting still. Muscle tonus comes from the activity of a small but changing number of motor units in a muscle; at any one time, some of the muscle's fibers are contracting and others are relaxed. Tonus is constantly being readjusted by the nervous system.

Muscle fiber types determine endurance and strength

Not all skeletal muscle fibers are alike, and a single muscle may contain more than one type of fiber. The two major types of skeletal muscle fibers are slow-twitch fibers and fast-twitch fibers (Figure 47.12a). Slow-twitch fibers are also called red muscle because they have lots of the oxygen-binding molecule myoglobin, they have lots of mitochondria, and they are well supplied with blood vessels. A single twitch of a slow-twitch fiber produces low tension.

The maximum tension a slow-twitch fiber can produce is low and develops slowly, but these fibers are highly resistant to fatigue. Because slow-twitch fibers have substantial reserves of fuel (glycogen and fat), their abundant mitochondria can maintain a steady, prolonged production of ATP if oxygen is available. Muscles with high proportions of slow-twitch fibers are good for long-term aerobic work (that is, work that requires lots of oxygen). Champion longdistance runners, cross-country skiers, swimmers, and bicyclists have leg and arm muscles consisting mostly of slow-twitch fibers (Figure 47.12b).

Fast-twitch fibers are also called white muscle because, in comparison to slow-twitch fibers, they have fewer mitochondria, little or no myoglobin, and fewer blood vessels. The white meat of domestic chickens is composed of fast-twitch fibers. Fast-twitch fibers can develop maximum tension more rapidly than slow-twitch fibers can, and that maximum tension is greater, but fast-twitch fibers become



Slow-twitch Fast-twitch

Cross-country skier

Long-distance runner Swimmer

Trained nonathletes

EFFECTORS: MAKING ANIMALS MOVE 841

Percent slow-twitch muscle (CZD) 20 40 60 80 100

—

Weight lifter

47.12 Two Types of Muscle Fibers

{a) Skeletal muscles stained with a reagent that shows slow-twitch fibers as dark. The upper photo shows muscle from a professional cyclist. The lower photo shows muscle from a nonathlete who has about 75% fast-twitch fibers; Sprinter this person would probably perform better as a sprinter than as a distance runner, (b) Athletes in different sports have different distributions of muscle fiber types.

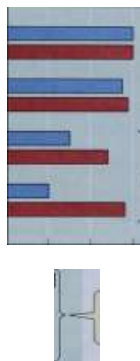
fatigued rapidly. The myosin of fast-twitch fibers has high ATPase activity, so they can put the energy of ATP to work very rapidly, but they cannot replenish it quickly enough to sustain contraction for a long time. Fast-twitch fibers are especially good for short-term work that requires maximum strength. Champion weight lifters and sprinters have leg and arm muscles with high proportions of fast-twitch fibers. What determines the proportion of fast- and slow-twitch fibers in your skeletal muscles? The most important factor is your genetic heritage, so there is some truth to the statement that champions are born, not made. To a certain extent, however, you can alter the properties of your muscle fibers through training. With aerobic training, the oxidative capacity of fast-twitch fibers can improve substantially. But a person born with a high proportion of fast-twitch fibers will never become a champion marathon runner, and a person born with a high proportion of slow-twitch fibers will never become a champion sprinter.

Skeletal Systems Provide Support for Muscles

Muscles can only contract and relax. Without something rigid to pull against, a muscle would just be a formless mass that twitches and changes shape. Skeletal systems provide rigid supports against which muscles can pull, creating directed movements. In this section, we'll examine the three types of skeletal systems found in animals: hydrostatic skeletons, exoskeletons, and endoskeletons.

A hydrostatic skeleton consists of fluid in a muscular cavity

The simplest type of skeleton is the hydrostatic skeleton of cnidarians, annelids, and many other soft-bodied invertebrates. It consists of a volume of incompressible fluid (water) enclosed in a body cavity surrounded by muscle.



Slow-twitch fibers are better adapted for sustained aerobic activity.

[Fast-twitch fibers can generate > ~ == ~> maximum tension quickly, but they also fatigue quickly.

20
40
60
80
100

Maximum oxygen uptake (I (ml/min/kg)

When muscles oriented in a certain direction contract, the fluid-filled body cavity bulges out in the opposite direction.

The sea anemone, a cnidarian (see Figure 31.6c), has a hydrostatic skeleton. Its body cavity is filled with seawater. To extend its body and its tentacles, the anemone closes its mouth and constricts muscle fibers that are arranged in circles around its body. Contraction of these circular muscles puts pressure on the water in the body cavity, and that pressure forces the body and tentacles to extend. The anemone retracts its tentacles and body by contracting muscle fibers that are arranged

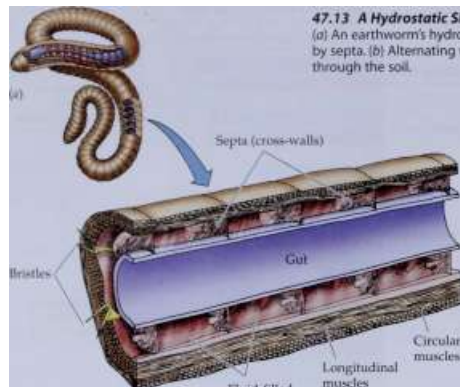
longitudinally in the body wall and along the tentacles.

An earthworm uses its hydrostatic skeleton to crawl. The earthworm's body cavity is divided into many separate, fluid-filled segments. The body wall surrounding each segment has two muscle layers: one in which the muscle fibers are arranged in circles around the body cavity, and another in which the muscle fibers run lengthwise (Figure 47.13a). If the circular muscles in a segment contract, the compartment in that segment narrows and elongates. If the lengthwise (longitudinal) muscles of a segment contract, the compartment shortens and bulges outward.

Alternating contractions of the earthworm's circular and longitudinal muscles create waves of narrowing and widening, lengthening and shortening, that travel down the body. The bulging, short segments serve as anchors as the narrowing, expanding segments project forward, and longitudinal contractions pull other segments forward. Bristles help the widest parts of the body to hold firm against the substrate (Figure 47.13b).

Another type of locomotion made possible by hydrostatic skeletons is the jet propulsion used by squid and octopuses.

842 CHAPTER FORTY-SEVEN



47.73 A Hydrostatic Skeleton

{a) An earthworm's hydrostatic skeleton consists of fluid-filled compartments separated by septa, (b) Alternating waves of elongation and contraction move the earthworm through the soil.

Constriction of circular muscles elongates the segments, pushing them forward.



Bristles anchor the segments to prevent backward sliding.



Bristles

Constriction of longitudinal muscles shortens the segments, pulling the trailing segments forward.

Fluid-filled compartments

Longitudinal muscles

Circular muscles

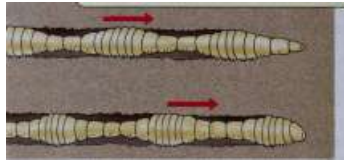
topuses. Muscles surrounding a water-filled cavity in these cephalopods contract, forcefully expelling water from the animal's body. As the water shoots out under pressure, the animal is propelled in the opposite direction.

Exoskeletons are rigid outer structures

An exoskeleton is a hardened outer surface to which muscles can be attached. Contractions of the muscles cause jointed segments of the exoskeleton to move relative to each other. The simplest example of an exoskeleton is the shell of a mollusk. Some marine mollusks, such as clams and snails, have shells composed of protein strengthened by crystals of calcium carbonate (a rock-hard material). These shells can be massive, affording significant protection against predators. The shells of land snails generally lack the hard mineral component and are much lighter. Mollusk shells can grow as the animal grows, and growth rings are usually apparent on the shells.

The most complex exoskeletons are found among the arthropods. An exoskeleton, or cuticle, covers all the outer surfaces of the arthropod's body and all its appendages. It is made up of plates secreted by a layer of cells just below the exoskeleton. A continuous, layered, waxy coating covers the entire body. The cuticle contains stiffening materials everywhere except at the joints, where flexibility must be retained. Muscles attached to the inner surfaces of the arthropod exoskeleton move its parts around the joints (Figure 47.14).

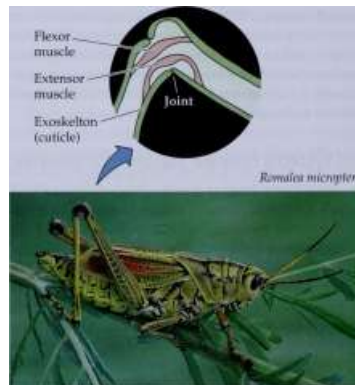
The layers of the cuticle include an outer, thin, waxy epi-cuticle that protects the bodies of terrestrial arthropods from drying out, and a thicker, inner endocuticle that forms most of the structure. The endocuticle is a tough, pliable material found only in arthropods. It consists of a complex of protein and chitin, a nitrogen-containing polysaccharide. In



marine crustaceans the endocuticle is further toughened by insoluble calcium salts. The thickness of the cuticle varies, but it can be thick enough to form a protective armor.

An exoskeleton protects all the soft tissues of the animal, but is itself subject to damage by abrasion and crushing. The greatest drawback of the arthropod exoskeleton is that

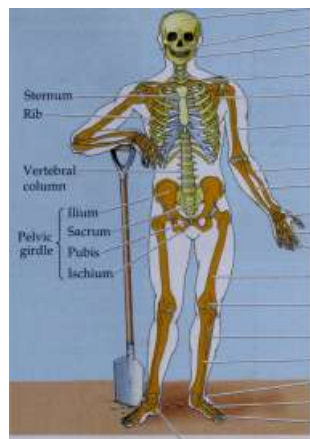
Flexor muscle



47.14 An Insect's Exoskeleton

Muscles attached to the exoskeleton of this lubber grasshopper move parts of the body around flexible joints.

Sternum Rib



Cranium

Maxilla Mandible

Skull

Clavicle -> p e c torial -Scapula J girdle

Humerus

Radius Ulna

Carpal bones Metacarpal bones Phalanges

Femur

Patella

Fibula Tibia

Tarsal bones — Metatarsal bones Phalanges Calcaneus J Axial skeleton ~] Appendicular skeleton

47.15 The Human Endoskeleton

Cartilage and bone make up the internal skeleton of a human being.

it cannot grow. Therefore, if the animal is to become larger, it must molt, shedding its exoskeleton and forming a new, larger one. A molting animal is vulnerable because the new exoskeleton takes time to harden. The animal's body is temporarily unprotected, and without a firm exoskeleton against which its muscles can exert maximum tension, it is unable to move rapidly. Soft-shelled crabs, a gourmet delicacy, are crabs caught when they are molting.

Vertebrate endoskeletons provide supports for muscles

The endoskeleton of vertebrates is an internal scaffolding to which muscles attach and against which they can pull. It is composed of rodlike, platelike, and tubelike bones, which are connected to each other at a variety of joints that allow a wide range of movements. Endoskeletons do not provide the protection that exoskeletons do, but their advantage is that they can grow. Because bones are inside the body, the body can enlarge without shedding its skeleton.

The human skeleton consists of 206 bones, some of which are shown in Figure 47.15. It can be divided into an axial skeleton, which includes the skull, vertebral column, and ribs, and an appendicular skeleton, which includes the pectoral girdle, the pelvic girdle, and the bones of the arms, legs, hands, and feet.

Two kinds of connective tissue cells produce large amounts of extracellular matrix material to create the vertebrate endoskeleton. The matrix material produced by cartilage cells is a rubbery mixture of proteins and polysaccharides. The principal protein in cartilage is collagen. Collagen

EFFECTORS: MAKING ANIMALS MOVE 843

fibers run in all directions through the gel-like matrix and give it the well-known strength and resiliency of "gristle."

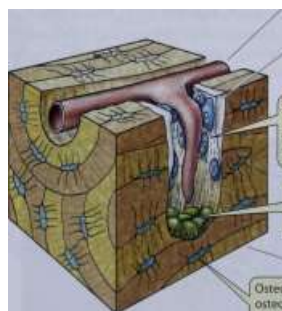
Cartilage is found in parts of the endoskeleton where both stiffness and resiliency are required, such as on the surfaces of joints, where bones move against each other. Cartilage is also the supportive tissue in stiff but flexible structures such as the larynx (voice box), the nose, and the ear pinnae. Sharks and rays are called cartilaginous fishes (see Figure 33.11) because their skeletons are composed entirely of cartilage. In all other vertebrates, cartilage is the principal component of the embryonic skeleton, but during development most of it is gradually replaced by bone.

Bone consists mostly of extracellular matrix material that contains collagen fibers as well as crystals of insoluble calcium phosphate, which give bone its rigidity and hardness. The skeleton serves as a reservoir of calcium for the rest of the body and is in dynamic equilibrium with soluble calcium in the extracellular fluids of the body. This equilibrium is under the control of calcitonin and parathyroid hormone (see Figure 41.10). If too much calcium is taken from the skeleton, the bones are seriously weakened.

The living cells of bone—called osteoblasts, osteocytes, and osteoclasts—are responsible for the dynamic remodeling of bone that is constantly under way (Figure 47.16). Osteoblasts lay down new matrix material on bone surfaces. These cells gradually become surrounded by matrix and eventually become enclosed within the bone, at which point they cease laying down matrix but continue to exist within small lacunae (cavities) in the bone. In this state they are called osteocytes. In spite of the vast amounts of matrix between them, osteocytes remain in contact with one another through long cellular extensions that run through tiny channels in the bone. Communication between osteocytes is important in controlling the activities of the cells that are laying down new bone or eroding it away.

Small blood vessel

Newly deposited bone matrix



Osteoblasts lay down new bone to fill tunnel dug out by osteoclasts.

Osteoclasts dig a tunnel through old bone.

Old bone

Osteocytes are osteoblasts that become trapped by their own handiwork.

47.76 Renovating Bone

Bones are constantly being remodeled by osteoblasts, which lay down bone, and osteoclasts, which dissolve bone.

844 CHAPTER FORTY-SEVEN

The cells that erode or reabsorb bone are the osteoclasts. They are derived from the same cell lineage that produces white blood cells. Osteoclasts burrow into bone, forming cavities and tunnels. Osteoblasts follow osteoclasts, depositing new bone. Thus the interplay of osteoblasts and osteoclasts constantly replaces and remodels the bones.

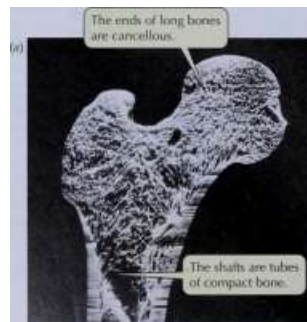
How the activities of the bone cells are coordinated is not understood, but stress placed on bones somehow provides them with information. A remarkable finding in studies of astronauts who had spent long periods in zero gravity was that their bones had decalcified. Conversely, certain bones of athletes can thicken during training, becoming considerably thicker than the same bones in nonathletes. Both thickening and thinning of bones are experienced by someone who has a leg in a cast for a long time. The bones of the uninjured leg carry the person's weight and thicken, while the bones of the inactive leg in the cast thin. The jawbones of people who lose their teeth experience less compressional force during chewing and become considerably reduced.

Bones develop from connective tissues

Bones are divided into two types on the basis of how they develop. Membranous bone forms on a scaffolding of connective tissue membrane. Cartilage bone forms first as a cartilaginous structure and is gradually hardened (ossified) to become bone. The outer bones of the skull are membranous bones; the bones of the limbs are cartilage bones.

Cartilage bones can grow throughout the ossification process. The long bones of the legs and arms, for example, ossify first at the centers and later at each end (Figure 47.17). Growth can continue until these areas of ossification join. The membranous bones forming the skull cap grow until their edges meet. The soft spot on the top of a baby's head is the point at which the skull bones have not yet joined.

The structure of bone may be compact (solid and hard) or cancellous (having numerous internal cavities that make it appear spongy, even though it is rigid). The architecture of a specific bone depends on its position and function, but most bones have both compact and cancellous regions. The shafts of the long bones of the limbs, for example, are cylindrical.

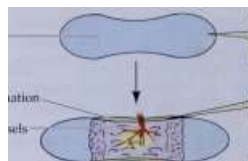


Cartilage

Bone formation

Blood vessels entering

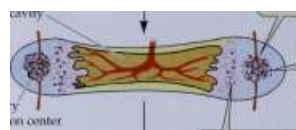
Marrow cavity



Long bones develop in the embryo as structures made of cartilage.

Ossification begins in the shaft.

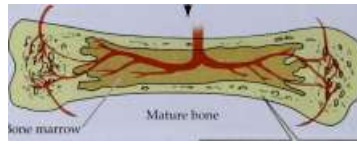
Secondary sites of ossification form at the ends.



Secondary ossification center

Blood vessels carry calcium and nutrients to developing bone.

Growth plates are sites of elongation between the ossified regions.



Bone marrow

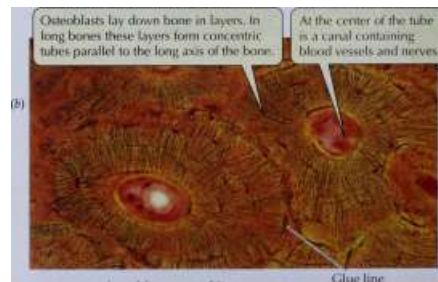
Eventually the areas of ossification fuse and elongation of the bone ceases.

47.17 The Growth of Long Bones

In the long bones of human limbs, ossification occurs first at the centers and later at each end.

Layers of compact bone surrounding central cavities that contain the bone marrow, where the cellular elements of the blood are made. The ends of the long bones are cancellous (Figure 47.18f). Cancellous bone is lightweight because of its numerous cavities, but it is also strong because its internal meshwork constitutes a support system. It can with-

At the center of the tube is a canal containing blood vessels and nerves.



47.18 Internal Architecture of Bone

(a) Bone may have cancellous ("with holes") and compact (solid) regions. (b) A micrograph of a section of a long bone shows Haversian systems with their central channels. Glue lines separate Haversian systems.

EFFECTORS: MAKING ANIMALS MOVE 845



47.19 Joints, Ligaments, and Tendons

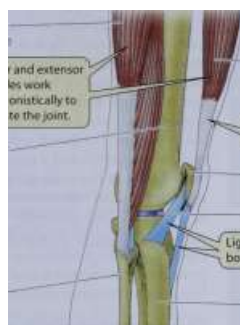
A side view of the knee shows the interactions of muscle, bone, cartilage, ligaments, and tendons at this crucial and vulnerable human joint.

Flexor -muscle

Flexor and extensor muscles work antagonistically to operate the joint.

Femur

Fibula



stand considerable forces of compression. The rigid, tubelike shaft of compact bone can withstand compression and bending forces. Architects and nature alike use hollow tubes as lightweight structural elements.

Most of the compact bone in mammals is called Haversian bone because it is composed of structural units called Haversian systems (Figure 47.18i>). Each Haversian system is a set of thin, concentric bony cylinders, between which are the osteocytes in their lacunae. Through the center of each Haversian system runs a narrow canal containing blood vessels (see Figure 47.16). Adjacent Haversian systems are separated by boundaries called glue lines. Haversian bone is resistant to fracturing because cracks tend to stop at glue lines.

Bones that have a common joint can work as a lever

Muscles and bones work together around joints, where two or more bones come together. Since muscles can only contract and relax, they create movement around joints by working in antagonistic pairs: When one contracts, the other relaxes. With respect to a particular joint, such as the knee, we can refer to the muscle that bends or flexes the joint as the flexor and the muscle that straightens or extends the joint as the extensor. The bones that meet at the joint are

Extensor

muscle

(quadriceps)

Tendons attach muscle to bone.

Patella (kneecap)

Cartilage

Ligaments attach bone to bone.

Tibia

held together by ligaments, which are flexible bands of connective tissue. Other straps of connective tissue, called tendons, attach the muscles to the bones (Figure 47.19). Ligaments help direct the forces generated by muscles by holding tendons in place. In many kinds of joints, only the tendon spans the joint, sometimes moving over the surfaces of the bone like a rope over a pulley. The tendon of the quadriceps muscle traveling over the knee joint is what is tapped to elicit the knee-jerk reflex (see Figure 46.4). The human skeleton has a wide variety of joints with different ranges of movement (Figure 47.20).

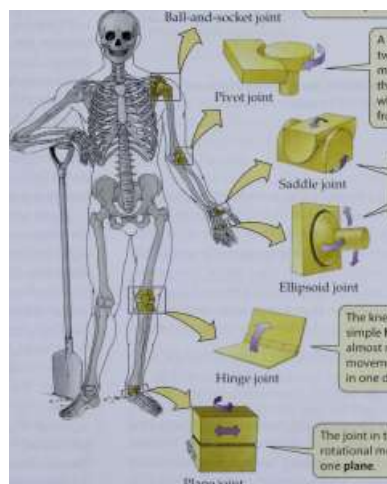
Bones move around joints and the muscles that work with those bones can be thought of as systems of levers. A lever has a power arm and a load arm that work around a fulcrum (pivot). The length ratio of the two arms determines whether a particular lever can exert a lot of force over a short distance or is better



Ball-and-socket joint

At the shoulders and hips are ball-and-socket joints

that allow movement in almost any direction.



A pivot joint where the two bones of the forearm meet at the elbow allows the smaller bone to rotate when the wrist is twisted from side to side.

j - ^ = ^ - 1 Several kinds of joints

permit some rotation, but not in all directions as the ball-and-socket joints do.

The knee joint is a simple hinge that has almost no rotational movement and can flex in one direction only.

The joint in the ankle allows rotational movement in only one plane.

rn

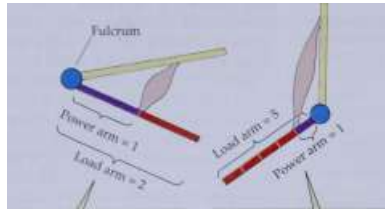


47.20 Types of Joints

The designs of joints are similar to mechanical counterparts and enable a variety of movements.

846 CHAPTER FORTY-SEVEN

Fulcrum



Lever system designed for power

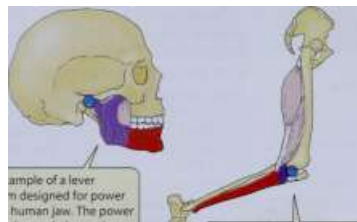
Load arm: power arm = 2:1 generates much force over a small distance.

Lever system designed for speed

Load arm: power arm = 5:1

moves low weights

long distances with speed.



An example of a lever system designed for power is the human jaw. The power arm is long relative to the load arm.

I

An example of a lever system designed for speed is the human leg. The power arm is short relative to the load arm.

47.21 Bones and Joints Work Like Systems of Levers

A lever system can be designed for power or speed.

at translating force into large or fast movements. Compare the jaw joint and the knee joint, for example (Figure 47.21). The power arm of the jaw is long relative to the load arm, allowing the jaw to apply great pressures over a small distance, as when you crack a nut with your teeth. The power arm of the lower leg, on the other hand, is short relative to the load arm, so you can run fast, jump high, and deliver swift kicks, but you cannot apply nearly the pressure with a leg that you can with your jaws.

Other Effectors

Muscles are universal in animals, but other effectors are more specialized and are shared by only a few animal species. Some specialized effectors are used for defense, some for communication, and some for capture of prey or avoidance of predators. In this section we mention only a few specialized effectors to give a sampling of their evolutionary diversity.

Nematocysts capture prey and repel predators

Some animals possess highly specialized organs that are fired like miniature missiles to capture prey and repel predators. Nematocysts are elaborate cellular structures produced only by hydras, jellyfishes, and other cnidarians. They are concentrated in huge numbers on the outer surface of the tentacles. Each nematocyst consists of a slender thread coiled tightly within a capsule, armed with a spinelike trigger projecting to the outside (see Figure 31.7). When potential prey brushes the trigger, the nematocyst fires, turning the thread inside out and exposing little spines along its base. The thread either entangles or penetrates the body of the victim, and a poison may be simultaneously released around the point of contact. Once the prey is subdued, it is pulled into the mouth of the cnidarian and swallowed. A jellyfish called the Portuguese man-of-war has tentacles that can be several meters long. These animals can capture, subdue, and devour full-grown

mackerel, and the poison of their nematocysts is so potent that it can kill a human who becomes tangled in the tentacles.

Chromatophores enable animals to change color

A change in body color is a response that some animals use to camouflage themselves in a particular environment or to communicate with other animals. Chromatophores are pigment-containing cells in the skin that can change the color and pattern of the animal. Chromatophores are under nervous or hormonal control, or both; in most cases they can effect a change within minutes or even seconds.

In squid, sole, and flounder, all of which spend much time on the seafloor, the famous chameleons (a group of African lizards; see Figure 33.19fr), and a few other animals, chromatophores enable the animal to blend in with the background on which it is resting and thus escape discovery by predators. Chromatophores with different pigments enable animals to assume different hues or to become mottled to match the background more precisely. In other mollusks, fishes, and lizards, a color change sends a signal to potential mates and territorial rivals of the same species.

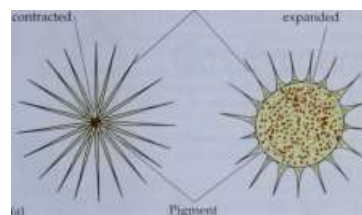
There are three principal types of chromatophore cells. The most common type has fixed cell boundaries, within which pigmented granules may be moved about by microfilaments. When the pigment is concentrated in the center of each chromatophore, the animal is pale; the animal turns darker when the pigment is dispersed throughout the cell. Another type of chromatophore is capable of amoeboid movement. These cells can mold themselves into shapes with a minimal surface area, leaving the tissue relatively pale, or they can flatten out to make the tissue appear darker.

The third type of chromatophore changes shape as a result of the action of muscle fibers radiating outward from the cell (Figure 47.22a). When the muscle fibers are relaxed, the chromatophores are small and compact, and the animal is pale. To darken the animal, the muscle fibers contract and spread the chromatophores over more of the body surface. These chromatophores can change so rapidly that they are

Chromatophore contracted

Muscle fibers

Chromatophore expanded



Pigment

47.22 Chromatophores Help Animals Camouflage Themselves or Communicate

(a) Muscle fibers around the chromatophores cause chromatophores to contract. (fc>) Cuttlefish are cephalopod mollusks that can change color patterns so fast that these changes can be used for rapid communication.

used in some species for communication during courtship and aggressive interactions. For example, the cuttlefish, a cephalopod, can signal courtship intentions to a potential mate on one side of its body while signaling aggressive threats to a rival on the other side of its body (Figure 47.22b).

Glands can be effectors

Glands are effector organs that produce and release chemicals. Some glands produce hormones for internal signaling. Other glands secrete substances into the gut or onto the body surface. Some of these secretions are used defensively or to capture prey. Others are pheromones, chemical signals released into the environment for communication with other individuals.

Certain snakes, frogs, salamanders, spiders, mollusks, and fishes have poison glands. Many of the poisons produced by these glands are extremely specific in their modes of action. For example, the poison dendrotoxin, which certain tribes of the Amazonian rainforest use on the tips of their arrows for hunting, comes from the skin of a frog and blocks certain potassium channels. The snake venom bun-garotoxin inactivates the neuromuscular acetylcholine receptors. The puffer fish poison tetrodotoxin blocks voltage-gated sodium channels. A poison from a mollusk, conotoxin, blocks calcium channels. Not all defensive secretions are poisonous, however. A well-known example is the odoriferous chemical mercaptan sprayed by skunks.

Electric organs can be shocking

Various fishes can generate electricity, as we saw in Chapter 45. These species include the electric eel, the knife fish, the torpedo (a type of ray), and the electric catfish. The electric fields they generate are used for sensing the environment, for communication, and also for stunning potential predators or prey. The electric organs of these animals evolved from muscles, and they produce electric potentials in the same general way as nerves and muscles do.



(b) *Sepia latimanu*

Electric organs consist of very large, disc-shaped cells arranged in long rows like stacks of batteries. When the cells discharge simultaneously, the electric organ can generate far more voltage and current than can nerve or muscle tissue. Electric eels, for example, can produce up to 600 volts with an output of approximately 100 watts--which is enough to light a row of light bulbs or to temporarily stun a person.

Chapter Summary

► Effectors enable animals to respond to information from their internal and external environments. Most effectors generate mechanical forces and cause movement.

Cilia, Flagella, and Cell Movement

► Cell movement is generated by two structures, microtubules and microfilaments, both of which consist of long protein molecules that can change their length or shape.

tubules and microfilaments, both of which consist of long protein molecules that can change their length or shape.

► The movements of cilia and flagella depend on microtubules. Review Figures 47.1, 47.3, 47.4

► Microfilaments allow animal cells to change their shape

and move.

Muscle Contraction

► The three types of vertebrate muscle are smooth, cardiac, and skeletal (striated). Review Figure 47.5

848 CHAPTER FORTY-SEVEN

► Smooth muscle provides contractile force for internal organs. Smooth muscle cells are electrically coupled through gap junctions, so action potentials that cause contraction spread rapidly throughout smooth muscle tissue. Autonomic neurotransmitters alter the membrane potential of smooth muscle cells. Review Figure 47.6

► The walls of the heart consist of sheets of branching cardiac muscle cells. The cells are electrically coupled through gap junctions, so that action potentials spread rapidly throughout sheets of cardiac muscle and cause coordinated contractions. Some cardiac muscle cells are pacemaker cells that generate the heartbeat.

► Skeletal, or striated, muscle consists of bundles of muscle fibers. Each muscle fiber is a huge cell containing multiple nuclei and numerous myofibrils, which are bundles of actin and myosin filaments. The regular, overlapping arrangement of the actin and myosin filaments into sarcomeres gives skeletal muscle its striated appearance. During contraction, the actin and myosin filaments slide past each other in a telescoping fashion. Review Figure 47.7

► The molecular mechanism of muscle contraction involves the binding of the globular heads of myosin molecules to actin. Upon binding, the myosin head changes conformation, causing the two filaments to move relative to each other. Release of the myosin heads from actin and their return to their original conformation requires ATP. Review Figure 47.8

► The plasma membrane of the muscle fiber is continuous with a system of T tubules that extends deep into the sarcoplasm (muscle cell cytoplasm). Review Figure 47.9

► When an action potential spreads across the plasma membrane and through the T tubules, it causes Ca^{2+} ions to be released from the sarcoplasmic reticulum. The Ca^{2+} ions bind to troponin and change its conformation, pulling the tropomyosin strands away from the myosin binding sites on the actin filament. Cycles of actin-myosin binding and release occur, and the muscle fiber contracts until the Ca^{2+} is returned to the sarcoplasmic reticulum. Review Figure 47.10

- ▶ In striated muscle, a single action potential causes a minimum unit of contraction called a twitch. Twitches occurring in rapid succession can be summed, thus increasing the strength of contraction. Review Figure 47.11
- ▶ Slow-twitch muscle fibers are adapted for extended, aerobic work; fast-twitch fibers are adapted for generating maximum forces for short periods of time. The ratio of slow-twitch to fast-twitch fibers in the muscles of an individual is genetically determined. Review Figure 47.12

Skeletal Systems Provide Support for Muscles

- ▶ Skeletal systems provide rigid supports against which muscles can pull.
- ▶ Hydrostatic skeletons are fluid-filled cavities that can be squeezed by muscles. Review Figure 47.13
- ▶ Exoskeletons are hardened outer surfaces to which internal muscles are attached. Review Figure 47.14
- ▶ Endoskeletons are internal, articulated systems of rigid rodlike, platelike, and tubelike supports consisting of bone and cartilage to which muscles are attached. Review Figure 47.15
- ▶ Bone is continually being remodeled by osteoblasts, which lay down new bone, and osteoclasts, which erode bone. Review Figure 47.16
- ▶ Bones develop from connective tissue membranes or from cartilage through ossification. Cartilage bone can grow until centers of ossification meet. Review Figure 47.17
- ▶ Bone can be solid and hard (compact bone), or it can contain numerous internal spaces (cancellous bone).
- ▶ Tendons connect muscles to bones; ligaments connect bones to each other and also help direct the forces generated by muscles by holding tendons in place. Review Figure 47.19
- ▶ Muscles and bones work together around joints as systems of levers. Review Figures 47.20, 47.21

Other Effectors

- ▶ Effector organs other than muscles include nematocysts, chromatophores, glands, and structures that produce electric pulses.

For Discussion

1. The amount of force a skeletal muscle can generate depends on its initial length. If the muscle is stretched or compressed prior to stimulation, it cannot generate maximum force of contraction. Why? Explain in terms of the molecular structure of the contractile mechanism.
2. If an intact axoneme is stimulated with Ca^{2+} and ATP, it flexes back and forth. However, if all of the proteins of the axoneme are enzymatically removed except for the microtubules and dynein, and the axoneme is then stimulated with Ca^{2+} and ATP, the microtubules telescope apart and the structure gets longer. Why?
3. Wombats are powerful digging animals, and kangaroos are powerful jumping animals. How do you think the structures of their legs would compare in terms of their designs as lever systems?
4. Why are ducks better long-distance fliers than chickens?
5. If an adolescent breaks a leg bone close to the ankle joint, after the break heals, that leg may not grow as long as the other one. Why?

48

Gas Exchange in Animals

3 ^^ In his book about his first ascent of Mt.

^^Mt. Everest, Sir John Hunt relates the following observation made at 26,000 feet, on the South Col, the last camp before the summit attempt. At this altitude climbers are almost totally incapacitated if they do not breathe supplemental oxygen from pressurized bottles:

And so back up the gradual slopes, the wind behind me. A much greater effort this, stopping every few yards with a slight anxiety lest I should not make the distance. As I approached the tents, I was astonished to see a bird, a chough, strutting about on the stones near me. ... During this day, too, Charles Evans saw what must have been a migration of small grey birds across the Col. Neither of us had thought to find any signs of life as high as this.

Birds in flight can consume oxygen at a rate that a well-trained human athlete cannot sustain for more than a few minutes. Yet birds fix over the summit of Everest, where human climbers must breathe supplemental oxygen just to plod along at a slow pace. How do they do it? Fish breathing water, with an oxygen content less than 5 percent that of air, can swim much faster, farther, and longer than the best human swimmer. How do they do it? The abilities of some animals to maintain high rates of metabolism depend in part on the capacities of their respiratory gas exchange systems.

Some animals carry out their respiratory gas exchange with water and others with air. Both water breathers and air breathers have respiratory systems with adaptations that maximize exchanges of oxygen and carbon dioxide with the environment. These adaptations include specialized surface areas where gas exchange takes place, breathing mechanisms that bring fresh air or water to those surfaces, and circulatory mechanisms for transporting respiratory gases to and from the internal sides of the gas exchange surfaces. Since gases cross the gas exchange surfaces by diffusion only, physical factors that limit diffusion determine the maximum capacities of gas exchange systems.

Flying High

Many birds can sustain the high metabolic costs of flight even at very high altitudes, where oxygen is scarce.

In this chapter we first describe the physical factors that influence respiratory gas exchange. Then we examine the respiratory gas exchange systems of a variety of species, including some highly efficient ones such as fish gills and bird lungs, and less efficient ones such as our own. We also look at the adaptations of the blood for transporting respiratory gases. Finally, we see how respiratory gas exchange systems are controlled and regulated.

Respiratory Gas Exchange

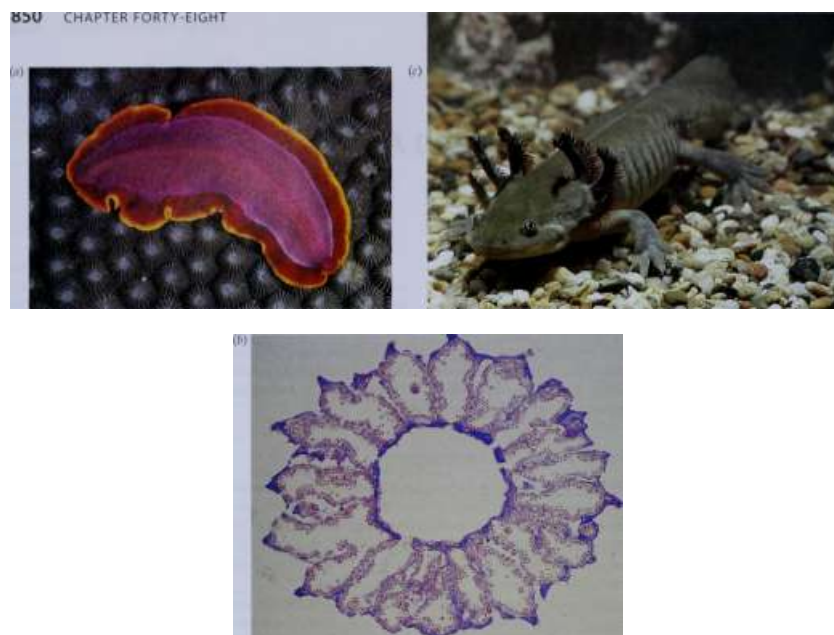
The respiratory gases are oxygen (O_2) and carbon dioxide (CO_2). Cells need to obtain O_2 from the environment to produce an adequate supply of ATP through the oxidation of nutrient molecules (see Chapter 7). An end product of the oxidative metabolism of nutrients is CO_2 which must be lost to the environment to prevent toxic effects.

Diffusion is the only means by which respiratory gases are exchanged between the internal body fluids of an animal and the outside medium—air or water. There are no active transport mechanisms to move respiratory gases across biological membranes. Because diffusion is a physical process, knowing the physical factors that influence rates of diffusion helps us understand the diverse adaptations of gas exchange systems. You might want to review what you learned about diffusion as a physical phenomenon in Chapter 5. Here, we discuss environmental factors that in-



•

850 CHAPTER FORTY-EIGHT



fluence diffusion rates, and then describe the adaptations of respiratory systems for facilitating the diffusion of respiratory gases.

Air is a better respiratory medium than water

O₂ can be obtained more easily from air than from water for several reasons.

► The oxygen content of air is much higher than the oxygen content of an equal volume of water. The maximum O₂ content of a rapidly flowing stream splashing over rocks and tumbling over waterfalls is less than 10 ml of O₂ per liter of water. The O₂ content of fresh air is about 200 ml of O₂ per liter of air.

► O₂ diffuses about 8,000 times more rapidly in air than in water. In a still pond, the O₂ content of the water can be zero only a few millimeters below the surface.

► When an animal breathes, it does work to move water or air over its specialized gas exchange surfaces. More energy is required to move water than air because water is 800 times more dense than air and about 50 times more viscous.

The slow diffusion of O₂ molecules in water is a problem for air-breathing animals as well as for water-breathing animals. Eukaryotic cells carry out cellular respiration in their mitochondria, which are located in the cytoplasm—an aqueous medium. Cells are bathed in extracellular fluid—

48.1 Keeping in Touch with the Medium

(a) No cell in the leaflike body of this marine flatworm is more than a millimeter away from seawater. (b) The same is true of sponges, which have body walls perforated by many channels lined with flagellated cells. These channels communicate with the outside world and with a central cavity. The flagella maintain currents of water through the channels, through the central cavity, and out of the animal. Every cell in the sponge is very close to the respiratory medium. (c) The gills of this newt project like a feathery fringe and provide a large surface area for gas exchange. Blood circulating through the gills comes into close contact with the respiratory medium.

also an aqueous medium. The slow rate of O₂ diffusion in water limits the efficiency of O₂ distribution from gas exchange surfaces to the sites of cellular respiration in both air-breathing and water-breathing animals.

Diffusion of O₂ in water is so slow that even animal cells with low rates of metabolism can be no more than a couple of millimeters away from a good source of environmental O₂. Therefore, in animals that lack an internal system for transporting O₂, no more than a few millimeters away from the outside world—ase^{el}ize^Tmit. One way some simple invertebrate animals have grown bigger in spite of this limit is to have a flat, leaflike body (Figure 48.1a). Another way is to have a very thin body built around a central cavity through which water circulates (Figure 48.1b). Otherwise, an animal must have specialized structures to provide an increased surface area for diffusion, an internal circulatory system to carry respiratory gases to and from these structures, and a way in which the surfaces of these gas exchange structures can be continuously bathed with fresh air or water (Figure 48.1c).

High temperatures create respiratory problems for aquatic animals

Animals that breathe water are in a double bind when environmental temperatures rise. Most water breathers are ectothermic—their body temperatures are closely tied to the temperature of the water around them. As the temperature of the water rises, so does their body temperature and metabolic rate (see Chapter 40 for a discussion of Q₁₀ relationships). Thus, with rising temperatures, water breathers

£ 3

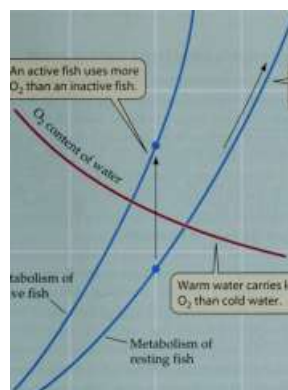
c o

u

c

O > X

o



The O_2 consumption of fish increases as water temperature increases.

Metabolism of active fish

Warm water carries less O_2 than cold water.

Metabolism of resting fish

"o c

QJ

C O

(J

c

QJ X

O

10 20 30

Water temperature ($^{\circ}C$)

40

48.2 The Double Bind of Water Breathers

Fish need more oxygen in warm water, but warm water carries less oxygen than cold water.

need more CV But warm water holds less dissolved oxygen gas than cold water does (just think of what happens when you open a warm bottle of soda). So, under conditions that increase the need these animals have for O_2 , there is less O_2 in their respiratory medium (Figure 48.2). In addition, if the animal performs work to move water across its gas exchange surfaces (as fish do, for example), the energy the animal must expend to breathe is

increased.

Therefore, as water temperature goes up, the water breather must extract

level, the pressure exerted by the atmosphere is equivalent to the pressure produced by a column of mercury 760 mm high. Therefore, barometric pressure (atmospheric pressure) at sea level is 760 mm of mercury (Hg). Because dry air is 20.9 percent O_2 , the partial pressure of oxygen (P_{O_2}) at sea level is 20.9 percent of 760 mm Hg, or about 159 mm Hg.

At higher elevations, where there is less air above, barometric pressure declines. At an altitude of 5,300 m barometric pressure is only half as much as it is at sea level, so the P_{O_2} at that altitude is only 80 mm Hg. At the summit of Mount Everest (8,848 m), the P_{O_2} is only about 50 mm Hg, roughly one-third what it is at sea level. Since the movement of O_2 across respiratory gas exchange surfaces and into the body depends on diffusion, its rate of movement depends on the P_{O_2} difference between the air and the body fluids. Therefore, the dramatically reduced P_{O_2} in the air at a high altitude constrains O_2 uptake. Because of these constraints, mountain climbers who venture to the heights of Mount Everest usually breathe O_2 from pressurized bottles (Figure 48.3).

Carbon dioxide is lost by diffusion

Respiratory gas exchange is a two-way process. CO_2 diffuses out of the body as O_2 diffuses in. Given the same partial pressure gradient, CO_2 and O_2 molecules diffuse at about the same rate, whether in air or in water. However, the partial pressure gradients for the diffusion of O_2 and CO_2 across gas exchange surfaces are not the same. The

Elevation

(meters)

12,000

11,000

10,000

9,000

more and more O_2 from its environment, and a lower percentage of that O_2 is available to support activities other than breathing.

O_2 availability decreases with altitude

Just as a rise in temperature reduces the supply of O_2 available to aquatic animals, an increase in altitude reduces the O_2 supply for air breathers, because the amount of O_2 in the atmosphere decreases with altitude.

One of the ways to express the amounts of gases in air and in water is by the partial pressures. At sea

8,000

7,000

6,000

5,000

4,000

3,000

2,000

1,000

Sea level

Air pressure (mm Hg)

- 100

-300



200



Concorde flights

Highest observed bird flight Mt. Everest

$P_{O_2} = 50 \text{ mmHg}$

48.3 Scaling Heights

The partial pressure of oxygen in the atmosphere decreases with altitude. Therefore, airplane cabins must be pressurized, and mountain climbers must carry pressurized containers of oxygen, at high altitudes. Birds, however, have been observed flying over even the highest peaks.

- 4000 ft < Mt. Kilimanjaro

$P_{O_2} = 80 \text{ mm Hg}$

15000 ft < Mt. Blanc

28000 ft < Mt. Whitney

35000 ft < Mt. Fuji

6000

Airplane cabins require pressurizing

700

- $P_{O_2} = 159 \text{ mmHg}$



852 CHAPTER FORTY-EIGHT

amount of CO_2 in the atmosphere is extremely low (0.03 percent), so there is always a good partial pressure gradient for loss of CO_2 from air-breathing animals.

Water-breathing animals are much more likely than air breathers to experience high partial pressures of CO_2 in their environments. If water is well aerated, well mixed, and does not contain a lot of dead organic material, the diffusion of CO_2 from an aquatic animal is not a problem. Stagnant waters that are home to much biological activity or rotting vegetation, however, can have high levels of CO_2 and not be able to support animal life. In both air breathers and water breathers, the need to transport CO_2 from where it is produced in the cells of the body to where it diffuses into the environment can be a limiting factor in gas exchange (and hence in metabolism).

Fick's law applies to all systems of gas exchange

All adaptations that maximize respiratory gas exchange influence one or more components of a simple equation called Fick's law of diffusion,

where

$$Q = DA \frac{C_1 - C_2}{L}$$

$Q =$

L

$\frac{C_1 - C_2}{L}$

- Q is the rate at which a substance such as O_2 diffuses between two locations
- D is the diffusion coefficient, which is a characteristic of the diffusing substance, the medium, and the temperature (for example, perfume has a higher D than motor oil, and substances diffuse faster in air than in water)
- A is the cross-sectional area over which the substance is diffusing
- C_1 and C_2 are the concentrations of the substance at two locations
- L is the distance between those locations

Therefore, $(C_1 - C_2)/L$ is a concentration gradient. In discussing respiratory gas exchange, we will use partial pressures rather than concentrations; this term will therefore become a partial pressure gradient.

Animals can maximize D for respiratory gases by using air rather than water as their gas exchange medium whenever possible. All other adaptations for maximizing respiratory gas exchange must influence the surface area for exchange or the partial pressure gradient across that surface area.

Respiratory Adaptations for Gas Exchange

Now that we know the factors that determine the rates of diffusion of respiratory gases, let's take a look at the fascinating array of adaptations that animals have evolved for respiratory gas exchange.

(a) Gills

Respiratory organs have large surface areas

Many anatomical adaptations maximize the specialized body surface area (A) over which respiratory gases can diffuse. External gills are highly branched and folded elaborations of the body surface that provide a large surface area for gas exchange with water (Figure 48.4c). External gills are found in larval amphibians and in many insect species. Because they consist of thin, delicate membranes, they minimize the length of the path (L) traversed by diffusing molecules of O_2 and CO_2 (see Figure 48.1c).

Because external gills are vulnerable to damage and are tempting morsels for carnivorous organisms, protective body

cavities for gills have evolved. Many mollusks, arthropods, and fishes have internal gills in such cavities.

Just as the gills of water breathers increase the surface area available for respiratory gas exchange, air-breathing vertebrates have enormous surface areas for gas exchange. Lungs are internal cavities for respiratory gas exchange with air. Their structure is quite different from that of gills (Figure 48.4b). Lungs have a large surface area because they are highly divided, and they are elastic so they can be inflated and deflated with air.

Most air-breathing invertebrates are insects, which have a unique respiratory gas exchange system consisting of a highly branched network of air-filled tubes called tracheae that branch through all the tissues of the insect's body. The terminal branches of these tubes are so numerous that they have an enormous surface area.

Ventilation and perfusion maximize partial pressure gradients

Fick's law of diffusion points to other possible adaptations besides increasing surface area that can increase respira-

(b) Lungs and tracheae

Gills are adaptations for gas exchange with water.

Lungs and tracheae are adaptations for gas exchange with air.

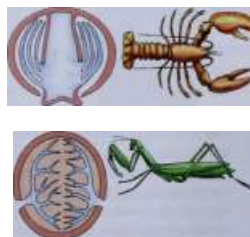


Lung



Internal gills

Tracheae



48.4 Gas Exchange Systems

A large surface area for the diffusion of respiratory gases is a common feature of animals.

torv gas exchange. Animals maximize the partial pressure gradients ($C_i - C_o/L$) that drive the diffusion of respiratory gases across their gas exchange membranes in several ways:

- Gill and lung membranes are very thin so that the path length for diffusion (L) is small.
- Breathing mechanisms ventilate the environmental side of the exchange surfaces so that they are exposed to fresh respiratory medium (air or water) with the highest possible partial pressure of O_2 and the lowest possible partial pressure of CO_2 .
- Circulatory systems perfuse the internal side of the exchange surfaces with a respiratory gas transport medium that helps maintain the lowest possible partial pressure of O_2 , and highest possible partial pressure of CO_2 , on the inside of the exchange membranes.

Air sacs

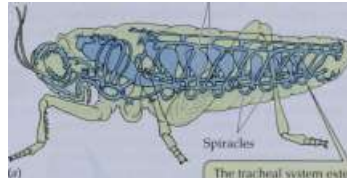
An animal's gas exchange system is made up of its gas exchange surfaces and the mechanisms it uses to ventilate and perfuse those surfaces. The following sections describe four gas exchange systems. First we look at the unique gas exchange system of insects. Then we describe two remarkably efficient systems: fish gills and bird lungs. Finally, we discuss mammalian lungs.

insect tracheae. Respiratory gases diffuse through air most of the way to and from every cell of an insect's body. This diffusion is achieved through a system of air tubes, or tracheae, that open to the outside environment through holes called spiracles in the sides of the abdomen (Figure 48.5a). The tracheae branch into even finer tubes, or tracheoles, until they end in tiny air capillaries (Figure 48.5c). In the insect's flight muscles and other highly active tissues, no mitochondrion is

more than a few micrometers away from an air capillary

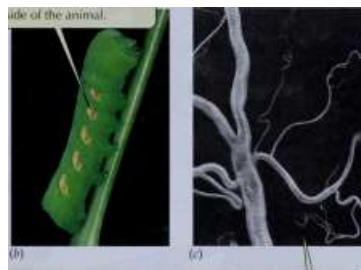
Because the diffusion rate of oxygen is so much higher in air than in water, air capillaries enable insects to supply oxygen to their cells at high rates. Many insects metabolize at high rates, but their relatively simple gas exchange systems are able to provide them with the oxygen they need. However, the rate of diffusion in insect tracheae and air capillaries is limited by their small diameter (A) and by the length (L) of these dead-end airways, so insects must be relatively small animals.

Some species of insects that dive and stay underwater for long periods make use of an interesting variation on diffusion. These insects carry with them a bubble of air. A small bubble may not seem like a very large reservoir of oxygen, yet these insects can stay underwater almost indefinitely with their small air supplies. The secret has to do with the P_{O_2} in the bubble. When the insect dives, the air bubble contains about 80 percent nitrogen and 20 percent O_2 . As the insect consumes the O_2 in its bubble, the bubble shrinks a little. The bubble doesn't disappear, however, because it consists mostly of nitrogen, which the insect does not consume. When the P_{O_2} in the bubble falls below the P_{O_2} in the surrounding water, O_2 diffuses from the water into the bubble. For these small animals, the rate of O_2 dif-



The spiracles of the larva of a spinx moth are arranged down the side of the anima

The tracheal system extends throughout the body and opens to the exterior through spiracles.



A scanning electron micrograph shows a trachea dividing into smaller trachioles, which give rise to still finer air capillaries.

48.5 The Tracheal Gas Exchange System of Insects

In insects, respiratory gases diffuse through a system of air tubes (tracheae) that open to the external environment through holes called spiracles.

fusion into the bubble is enough to meet their O_2 demand while they are underwater.

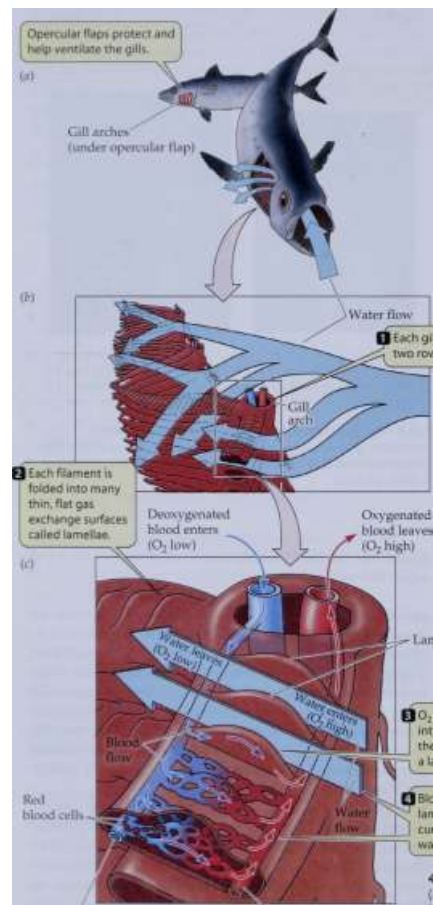
fish gills. The internal gills of fishes are supported by usually four gill arches on either side of the fish and lie between the mouth cavity and the protective opercular flaps (Figure 48. 6a). Water flows unidirectionally into the fish's mouth, over the gills, and out from under the opercular flaps, so that the gills are continuously bathed with fresh water. This constant flow of water moving over the gills maximizes the P_{O_2} on the external surfaces. On the internal side, the circulation of blood minimizes the P_{O_2} by sweeping the O_2 away as rapidly as it diffuses across.

The gills have an enormous surface area for gas exchange because they are so highly divided. Each gill consists of hundreds of leaf-shaped gill filaments (Figure 48.6i>). The upper and lower flat surfaces of each gill filament have rows of evenly spaced folds, or lamellae. The lamellae are the gas exchange surfaces. Their delicate structure minimizes the path length for diffusion of gases between blood and water. The surfaces of the lamellae consist of highly

854 CHAPTER FORTY-EIGHT

flattened epithelial cells, so the water and the red blood cells are separated by little more than 1 or 2 μm .

The flow of blood perfusing the inner surfaces of the lamellae, like the flow of water over the gills, is unidirectional. Afferent blood vessels bring blood to the gills, while efferent blood vessels take blood away from the gills (Figure i. Blood flows through the lamellae in the direction op-



Each gill arch supports two rows of gill filament

opposite to the flow of water over the lamellae. This counter-current flow maximizes the P_{50} gradient between water and blood, making gas exchange more efficient than it would be in a system using concurrent (parallel) flow (Figure 48.7).

Some fish, including anchovies, tuna, and certain species of sharks, ventilate their gills by swimming almost constantly with their mouths open. Most fish, however, ventilate the external surfaces of their gills by means of a two-pump mechanism that maintains a unidirectional and constant flow of water over the gills. The closing and contracting of the mouth cavity pushes water over the gills, and the opening and closing of the opercular flaps pulls water over the gills.

In summary, fish can extract an adequate supply of O_2 from meager environmental sources by maximizing the surface area for diffusion, minimizing the path length for diffusion, and maximizing oxygen extraction efficiency by means of constant, unidirectional, countercurrent flow of blood and water over the opposite sides of their gas exchange surfaces.

bird lungs. Birds can sustain extremely high levels of activity for much longer than mammals can—even at very high altitudes where mammals cannot even survive. Yet the lungs of a bird are smaller than the lungs of a

Lamellae

Red blood cells

similar-sized mammal. How can this be? Bird lungs have a unique structure that allows air to flow unidirectionally through the lungs, rather than having to flow in and out through the same airways, as it does in mammalian lungs.

In addition to lungs, birds have air sacs at several locations in their bodies. The air sacs are interconnected with the lungs and with air spaces (another unique feature of birds) in some of the bones (Figure 48.8). The air sacs receive inhaled air, but they are not gas exchange surfaces. The composition of air in an air sac does not change rapidly, as it would if O_2 were diffusing into the blood and CO_2 were diffusing into the air sac.

As in other air-breathing vertebrates, air enters and

leaves a bird's gas exchange system through a trachea

(commonly known as the windpipe), which divides into

smaller airways called bronchi (singular bronchus). In

air-breathing vertebrates other than birds,

the bronchi generate trees of branching

airways that become finer and finer until

they dead-end in clusters of microscopic, membrane-enclosed air sacs, where gases are exchanged. In bird lungs, however, there are no dead ends; air flows unidirectionally through the lungs. O_2 diffuses from water into the blood over the entire length of a lamella.

Blood flow through the lamellae is counter-current to the flow of water over the lamellae. tionally through the lungs.

/

Afferent blood vessel

Efferent blood vessel

48.6 Fish Gills

(a) Water flows unidirectionally over the gills of a fish, (b) Gill filaments have a large surface area and thin membranes, (c) Blood flows through the lamellae in the direction opposite (left to right, in this depiction) to the flow of water (right to left) over the lamellae.

(a) Concurrent flow

% Saturation Gill lamellae

Blood flow 20% 30%

50% 50% 50% 50% 50% 50%

$r =$

Water flow 100% 80% 70% 60% 50% 50% 50% 50% 50% 50% 50% 50% 50%

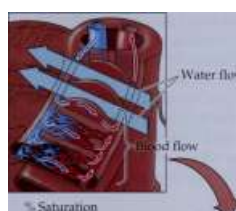
W k.

Blood flow 20%

0% 50% 50% 50% 50% 50% 50% 50% 50%

(b) Countercurrent flow

Water flow



% Saturation

p k

Blood flow 20% 25% 30% 35% 40% 45% 50% 55% 60% 65% 70% 75% 100%

*. r

GAS EXCHANGE IN ANIMALS 855

48.7 Countercurrent Exchange Is More Efficient than Concurrent Exchange

In these models of concurrent and countercurrent exchange, the numbers represent the oxygen saturation of blood and water, (a) In the concurrent exchanger, the percentages of saturation of blood and water reach equilibrium even before the water has flowed halfway across the exchange surface. (b) There is more complete exchange in the case of countercurrent flow because the water is always more saturated than the blood as it passes over the exchange surface, so that a gradient of O_2 saturation is always maintained.

avian lungs do. To make things even more puzzling, bird lungs contract during inhalation and expand during exhalation!

The puzzle of how birds breathe was solved by an experiment that placed small oxygen sensors at different locations in the air sacs and airways of birds. The bird could then be exposed to pure O_2 , and the progress of that single breath through the bird's gas exchange

m

Water flow 25% 30% 35% 40% 45% 50% 55% 60% 65% 70% 75% 80% 100%

Blood flow 20% 25% 30% 35% 40% 45% 50% 55% 60% 65% 70% 75% 100%

V

In bird lungs, the bronchi divide into tubelike parabronchi (Figure 48.9). Running between the parabronchi are tiny airways called air capillaries. Air flows through the lungs in the parabronchi, but crosses between parabronchi through the air capillaries. The air capillaries are the gas exchange surfaces. They are tiny but numerous, so they provide an enormous surface area for gas exchange.

Another unusual feature of bird lungs is that they expand and contract less during a breathing cycle than mam-



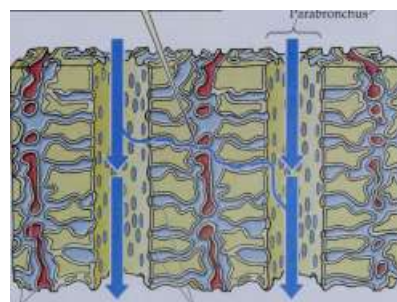
Air capillaries carry air from a parabronchus, over blood capillaries where O_2 is absorbed, and out through other parabronchi.



test*



Parabronchus^



Blood capillaries

Air capillaries

Trachea

48.8 The Respiratory System of a Bird

The air sacs and air spaces in the bones are unique to birds.

48.9 Air Flows through Bird Lungs Constantly and Unidirectionally

The gas exchange surfaces of birds are air capillaries branching off the parabronchi, which run through the lungs.

856 CHAPTER FORTY-EIGHT

EXPERIMENT

Question: How does air flow through a bird's respiratory system?

METHOD Place oxygen sensors at different locations in a

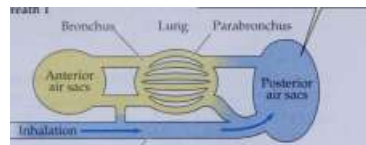
bird's respiratory system. Give the bird one breath of pure oxygen, followed by a breath of normal air. Record when oxygen reaches different sensors.

RESULTS Breath 1

f

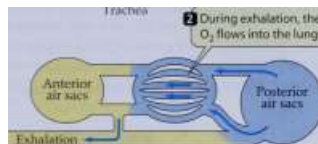
The breath of pure O_2 is inhaled directly to the posterior air sacs.

Bronchus



Trachea

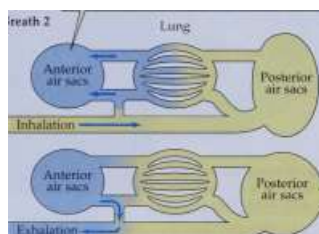
During exhalation, the pure O_2 flows into the lungs.



f

During the next inhalation, the breath of pure O_2 flows from the lungs to the anterior air sacs.

Breath 2



Finally, during the next exhalation, the breath marked by pure O_2 is expelled.

Conclusion: Air travels through the lungs in one direction, from the posterior to the anterior air sacs. Two cycles of inhalation and exhalation are required for the air to travel through the bird's respiratory tract.

48.70 The Path of Air Flow through Bird Lungs *

The fresh air a bird takes in with one breath (blue) travels through the lungs in one direction, from the posterior to the anterior air sacs. Two cycles of inhalation and exhalation are required for the air to travel the full length of the bird's respiratory system.

This system could be followed by the oxygen sensors. This experiment showed that a single breath remains in the bird's gas exchange system for two cycles of inhalation and exhalation, and that the air sacs work as bellows maintaining a



continuous and unidirectional flow of fresh air through the lungs (Figure 48.10).

The advantages of the bird gas exchange system are similar to those of fish gills. Because the air sacs keep fresh air from the outside flowing unidirectionally and practically continuously over the gas exchange surfaces, the P_{O_2} on the environmental side of those surfaces is maximized. Furthermore, the unidirectional flow of air through the system makes possible a pattern of blood flow, to minimize the P_{O_2} on the internal side of the exchange surfaces.

It is now clear how birds can live over Mount Everest. A bird is able to supply its gas exchange surfaces with a continuous flow of fresh air that has a P_{O_2} close to that of the ambient air. Even when the P_{O_2} of the ambient air is only slightly above the P_{O_2} of the blood, O_2 can diffuse from air to blood. Next we will see why humans find it difficult to sustain even low levels of metabolic activity at such high altitudes.

tidal breathing in mammals. At the beginning of their evolution, lungs were dead-end sacs, and they remain so today in all air-breathing vertebrates except birds. Because lungs are dead-end sacs, ventilation cannot be constant and unidirectional, but must be tidal: Air flows in and out by the same route.

Figure 48.11 shows how we use our lung capacity in breathing (Figure 48.11). When we are at rest, the amount of air that moves in and out of the lungs during one normal breath is called the tidal volume (about 500 ml for an average human adult). We can breathe much more deeply and inhale more air than our resting tidal volume; the additional volume of air we can take in above normal tidal volume is our inspiratory reserve volume. Conversely, we can forcefully exhale more air than we normally do during a resting exhalation. This additional amount of air that can be forced out of the lungs is the expiratory reserve volume. But even after the most extreme exhalation possible, some air remains in the lungs. The lungs and airway cannot be collapsed completely; they always contain a residual volume. The total lung capacity is the sum of the residual volume, expiratory reserve volume, tidal volume, and inspiratory reserve volume.

Tidal breathing severely limits the partial pressure gradient available to drive the diffusion of O_2 from air into the blood. Fresh air is not moving into the lungs during half of the respiratory cycle; therefore, the average P_{O_2} of air in the lungs is considerably less than it is in the air outside the lungs. Furthermore, the incoming air mixes with the stale air that was not expelled by the previous exhalation. The lung volume that is not ventilated with fresh air is called dead space. This dead space consists of the residual volume and, depending on the depth of breathing, some or all of the expiratory reserve volume.

The scale in Figure 48.11 tells us that a tidal volume of 500 ml of fresh air mixes with up to 2,000 ml of stale air before reaching the gas exchange surfaces in our lungs. When the P_{O_2} in the ambient air is 150 mm Hg, the P_{O_2} of the air that reaches our gas exchange surfaces is only about 100

*

RESEARCH METHOD

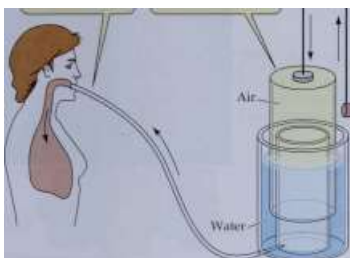
Breathing into a spirometer allows characteristics of breathing to be measured.

A spirometer contains a trapped reservoir of air and can measure changes in its volume.



"^

Spirometer



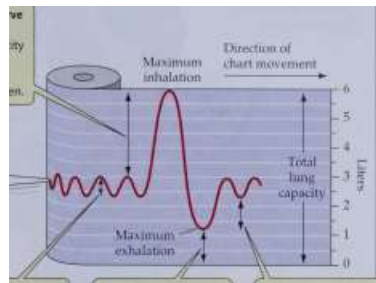
Inspiratory reserve volume is an additional capacity of the lungs to allow a deep breath to be taken.

Pen

Direction of Maximum chart movement inhalation * ~

Tidal volume is the

normal amount of air exchanged in breathing when at rest.



43 R

Residual volume is

the amount of air left in the lungs after maximum exhalation.

Expiratory reserve volume is the

additional air that can be forcefully exhaled.



48.11 Measuring Lung Ventilation with a Spirometer

Breathing from a closed reservoir of air and measuring the changes in the volume of that reservoir demonstrates the characteristics of mammalian tidal breathing.

mm Hg. By contrast, the P_{O_2} in the water that bathes the lamellae of fish gills or in the air that flows through the air capillaries of bird lungs is the same as the P_c in the outside water or air.

In addition to reducing the partial pressure gradient, tidal breathing reduces the efficiency of gas exchange in another way: It does not allow countercurrent gas exchange between air and blood. Because air enters and leaves the gas exchange structures by the same route, there is no anatomical way that blood can flow countercurrent, or even cross current, to their flow.

Mammalian Lungs and Gas Exchange

To offset the inefficiencies of tidal breathing, mammalian lungs have some design features that maximize the rate of gas exchange: an enormous surface area, and a very short path length for diffusion. Mammalian lungs serve the respiratory needs of mammals well, considering the ecologies and lifestyles of these animals.

Air enters the lungs through the oral cavity or nasal passage, which join together in the pharynx (Figure 48.12). From the pharynx, the esophagus conducts food to the stomach and a single airway leads to the lungs. At the beginning of this airway is the larynx, or voice box, which houses the vocal cords. The larynx is the "Adam's apple" that you can see or feel on the front of your neck. The major airway, the trachea, has a diameter of about 2 cm. The thin walls of the trachea are prevented from collapsing by rings of cartilage that support them as air pressure changes during the breathing cycle. If you run your fingers

down the front of your neck just below your larynx, you can feel a couple of these rings of cartilage.

The trachea branches into two smaller bronchi, one leading to each lung. The bronchi branch repeatedly to generate a treelike structure of progressively smaller airways extending to all regions of the lungs (see Figure 48.12). As the branching of the bronchial tree continues to produce still smaller airways, the cartilage supports eventually disappear, marking the transition to bronchioles. The branching continues until the bronchioles are smaller than the diameter of a pencil lead, at which point tiny, thin-walled air sacs called alveoli begin to appear.

The alveoli are the sites of gas exchange. Because the airways only conduct air to and from the alveoli and do not themselves conduct gas exchange, their volume is physiologically small. The number of alveoli in human lungs is about 300 million. Even though each alveolus is very small, the combined surface area for diffusion of respiratory gases is about 70 m²—the size of a badminton court.

Each alveolus is made of very thin cells. Between and surrounding the alveoli are networks of the smallest of blood vessels, the capillaries, whose walls are also made up of exceedingly thin endothelial cells. Where capillary meets alveolus, very little space separates them (see Figure 48.12), so the length of the diffusion path between air and blood is less than 2 μ m. Even the diameter of a red blood cell is greater—about 7 μ m.

Respiratory tract secretions aid breathing

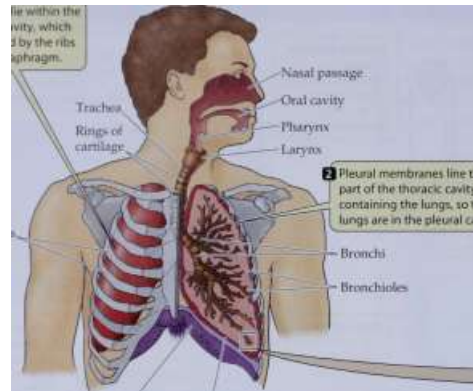
Mammalian lungs have two other important adaptations that do not directly influence their gas exchange properties:

858 CHAPTER FORTY-EIGHT

[The lungs lie within the thoracic cavity, which is bounded by the ribs and the diaphragm.]

Nasal passage Oral cavity

Ribs



| The bronchi are the major air passageways of the lungs. They lead to the bronchioles, which are finely branched.

Pleural membranes line the part of the thoracic cavity containing the lungs, so the lungs are in the pleural cavities.



Diaphragm Pleural cavity

48.12 The Human Respiratory System

The diagram traces the hierarchy of human respiratory structures from the lungs down to the minuscule alveoli.

the production of mucus and the production of surfactant.

A surfactant is a substance that reduces the surface tension of a liquid. Lung surfactant reduces the surface tension of the film of fluid lining the insides of the alveoli. What is surface tension and how does it affect lung function? Surface tension is a result of cohesion between the molecules of a liquid. Cohesion gives the surface of the liquid the properties of an elastic membrane. Surface tension is what allows some insects to walk on the surface of water (see Figure 2.16). A surfactant interferes with the cohesive forces that create surface tension. Detergent is a surfactant, and when added to water, it makes walking on water difficult for the water strider.

The thin, aqueous layer that lines the alveoli has surface tension, which must be overcome to inflate the lungs. Surface tension normally is reduced by surfactant molecules produced by certain cells in the alveoli. If a baby is born more than a month prematurely, however, these cells may not yet be producing surfactant. Such a premature baby has great difficulty breathing because an enormous effort is required to stretch the alveoli against the surface tension. This condition, known as respiratory distress syndrome, may cause the baby to die from exhaustion and suffocation. Common treatments have been to put the baby on a respirator to assist its breathing and to give the baby hormones to speed its lung development. A new approach, however, is to apply surfactant to the lungs via an aerosol.

Many cells lining the airways produce a sticky mucus that captures bits of dirt and microorganisms that are inhaled. This mucus must be continually cleared from the air-

ways. Other cells lining the airways have cilia (see Figure 47.2) whose beating moves the mucus with its trapped debris up toward the pharynx, where it is swallowed. This phenomenon, called the mucus escalator, can be adversely affected by inhaled pollutants. Smoking one cigarette a day can immobilize the cilia of the airways for hours. A smoker's cough results from the need to clear the obstructing mucus from the airways when the mucus escalator is out of order.

Lungs are ventilated by pressure changes in the thoracic cavity

As Figure 48.12 shows, human lungs are suspended in the thoracic cavity, which is bounded on the top by the shoulder girdle, on the sides by the rib cage, and on the bottom by a domed sheet of muscle, the diaphragm. The thoracic cavity is lined on the inside by the pleural membranes, which divide it into right and left pleural cavities enclosing each lung. Because the pleural cavities are closed spaces, any effort to increase their volume creates negative pressure—suction—inside them.

Negative pressure within the pleural cavities causes the lungs to expand as air flows into them from the outside. This is the mechanism of inhalation. The diaphragm contracts to begin an inhalation. This contraction pulls the diaphragm down, increasing the volume of the thoracic and pleural cavities (Figure 48.13). As pressure in the pleural cavities becomes more

negative, air moves into the lungs. Exhalation begins when the contraction of the diaphragm ceases. The diaphragm relaxes and moves up, and the elastic recoil of the lungs pushes air out through the airways. During tidal breathing, inhalation is an active process and exhalation is a passive process.

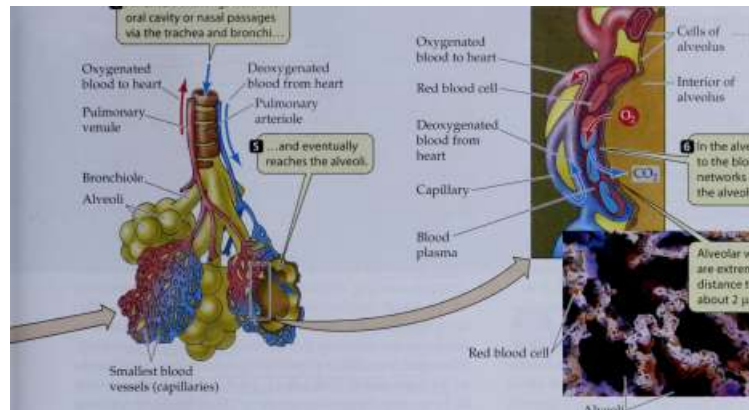
The diaphragm is not the only muscle that changes the volume of the pleural cavities. Between the ribs are two sets

GAS EXCHANGE IN ANIMALS 859

Q Air enters the lungs from the oral cavity or nasal passages via the trachea and bronchi.

Cells of alveolus

Interior of alveolus



In the alveoli, the air is very close to the blood flowing through the networks of capillaries surrounding the alveoli.

Alveolar walls and capillary walls are extremely thin, minimizing the distance that O_2 must diffuse to about 2 μm .

Smallest blood vessels (capillaries)

Alveoli

+2 +1

Inhalation

Exhalation

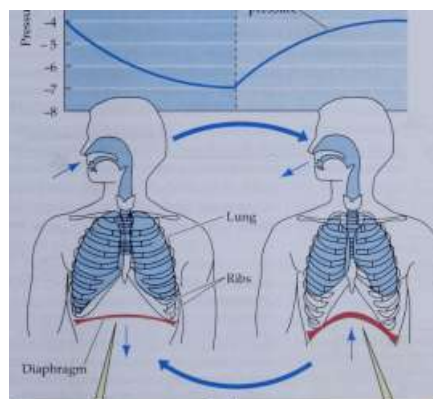
X



I -i

Alveolar' pressure

Pleural cavity pressure^



During inhalation, the diaphragm contracts, muscles between the ribs contract (thereby elevating them), and the pleural cavities expand, sucking air in.

During exhalation, the diaphragm relaxes, muscles between the ribs relax (thereby lowering them), and the pleural cavities contract, pushing air out.

of intercostal muscles. The external intercostal muscles expand the pleural cavities by lifting the ribs up and outward. The internal intercostal muscles decrease the volume of the thoracic cavity by pulling the ribs down and inward. When heavy demands are placed on the gas exchange system, such as during strenuous exercise, the external intercostal muscles increase the volume of air inhaled, making use of the inspiratory reserve volume, and the internal intercostal muscles increase the amount of air exhaled, making use of the expiratory reserve volume.

When the diaphragm is at rest between tidal breaths, the pressure in the pleural cavities is still slightly negative. This slight suction keeps the alveoli partly inflated. If the thoracic wall is punctured—by a knife wound, for example—air leaks into the pleural cavity, and the pressure from this air causes the lung to collapse. If the hole in the thoracic wall is not sealed, the breathing movements of the diaphragm and intercostal muscles pull air into the pleural cavity rather than into the lung, and ventilation of the alveoli in that lung ceases.

48.13 Into the Lungs and Out Again

Inhalation is an active process spurred by the contraction of the diaphragm. Exhalation generally is a passive process as the diaphragm relaxes. During inhalation, the negative pressure in the pleural cavity increases, expanding the elastic lung tissue and sucking air into the lungs. During exhalation, the negative pressure in the pleural cavity decreases, allowing the elastic lung tissue to recoil to create a positive pressure in the lungs and expel air.



860 CHAPTER FORTY-EIGHT

Blood Transport of Respiratory Gases

The circulatory system is the subject of the next chapter, but since two of the substances the blood transports are the respiratory gases (O_2 and CO_2), we must discuss blood here. The circulatory system uses a pump (the heart) and a network of blood vessels to transport blood and the substances it carries around the body. As O_2 diffuses across the gas exchange surfaces into the blood vessels, the circulating blood sweeps it away. As we have seen, this internal perfusion of the gas exchange surfaces minimizes the P_{pO_2} on the internal side and promotes the diffusion of O_2 across the surface at the highest possible rate. The blood then delivers this O_2 to the cells and tissues of the body.

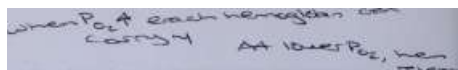
The liquid part of the blood, the blood plasma, carries some O_2 in solution, but its ability to transport O_2 is quite limited. Blood plasma can carry only about 0.3 ml of oxygen per 100 ml, which is inadequate to support the metabolism of a person at rest. Fortunately, the blood also contains red blood cells, which are red because they are loaded with the oxygen-binding pigment hemoglobin. Hemoglobin increases the capacity of blood to transport oxygen by about 60-fold. There is quite a diversity of O_2 -binding pigments among the animals; there is even considerable diversity of hemoglobin molecules. The discussion that follows focuses on human hemoglobin.

Hemoglobin combines reversibly with oxygen

Red blood cells contain enormous numbers of hemoglobin

molecules. Hemoglobin is a protein consisting of four polypeptide subunits (see Figure 3.7). Each of these polypeptides surrounds a heme group—an iron-containing structure that can reversibly bind a molecule of O_2 .

As O_2 diffuses into the red blood cells, it binds to hemoglobin. Once O_2 is bound, it cannot diffuse back across the red cell plasma membrane. By mopping up O_2 molecules as they enter the red blood cells, hemoglobin maximizes the partial pressure gradient driving the diffusion of O_2 into the cells. In addition, it enables the red blood cells to carry a large amount of O_2 to the tissues of the body.



t

The normal P_{aO_2} of deoxygenated blood is 40 mm Hg. The P_{aO_2} of blood leaving the lungs is about 100 mm Hg.

100

75

250

~Y



lungs is about 100 mm Hg.

O_2

The ability of hemoglobin to pick up or release O_2 depends on the partial pressure of O_2 in its environment. When the P_{O_2} of the blood plasma is high, as it usually is in the lung capillaries, each molecule of hemoglobin can carry its maximum load of four molecules of O_2 . As the blood circulates through the rest of the body, it encounters lower P_{O_2} values. At these lower P_{O_2} values, the hemoglobin releases some of the O_2 it is carrying (Figure 48.14).

As you can see from the figure, the relation between P_{O_2} and the amount of O_2 bound to hemoglobin is not linear, but S-shaped (sigmoid). The sigmoid hemoglobin- O_2 binding curve reflects interactions between the four subunits of the hemoglobin molecule, each of which can bind one molecule of O_2 . At low P_{O_2} values, only one subunit will bind an O_2 molecule. When it does so, the shape of this subunit changes, causing an alteration in the quaternary structure of the whole hemoglobin molecule (see Chapter 3). This structural change makes it easier for the other subunits to bind a molecule of O_2 ; that is, their O_2 affinity is increased. Therefore a smaller increase in P_{O_2} is necessary to get most of the hemoglobin molecules to bind two O_2 molecules (that is, to become 50 percent saturated) than it was to get them to bind one molecule of O_2 (25 percent saturated). The influence of the binding of O_2 by one subunit on the O_2 affinity of the other subunits is called positive cooperativity, because

binding of the first molecule makes binding of the second easier, and so forth.

Once the third molecule of O_2 is bound, however, the relationship seems to change, as a larger increase in P_{O_2} is required to reach 100 percent saturation. This upper bend of the sigmoid curve is due to a probability phenomenon: The closer we get to having all subunits occupied, the less likely it is that a single O_2 molecule will find a place to bind. Therefore it takes a relatively large P_{O_2} to achieve 100 percent saturation.

This is a good place to mention the danger posed by carbon monoxide (CO), which can come from a faulty furnace or from combusting a fuel such as charcoal or kerosene without adequate ventilation. CO binds to hemoglobin with a higher affinity than does O_2 . Thus, CO destroys the ability of hemoglobin to transport and release O_2 to the tissues of the body. The victim loses consciousness and can die because the brain lacks O_2 .

$t > 0$

>

25



Of the O_2 in arterial blood, 25% is released to tissues during normal metabolism.

LJ

An oxygen reserve of 75% is held by the hemoglobin and can be released to tissues

with a low P_{O_2} .

20

40 60 80 P_{O_2} (mm Hg)

100

48.74 The Binding of Oxygen to Hemoglobin Depends on the P_{O_2}

Hemoglobin in blood leaving the lungs is 100 percent saturated (four molecules of O_2 are bound to each hemoglobin). Most hemoglobin molecules will drop only one of their four O_2 molecules as they circulate through the body, and are still 75 percent saturated when the blood returns to the lungs. The steep portion of this oxygen-binding curve comes into play

when tissue P_{O_2} falls below the normal 40 mm Hg. At this point the hemoglobin will "unload" its oxygen reserves.



The O_2 -binding properties of hemoglobin help get O_2 to the tissues that need it most. In the lungs, where the P_{O_2} is about 100 mm Hg, the hemoglobin is 100 percent saturated. The P_{O_2} in blood returning to the heart from the body is usually about 40 mm Hg. You can see from Figure 48.14 that at this P_{O_2} , the hemoglobin is still about 75 percent saturated. This

means that as the blood circulates around the body, only about 1 of 4 O₂ molecules it carries is released to the tissues. That seems inefficient, but it is really quite adaptive, because the hemoglobin keeps 75 percent of its oxygen in reserve to meet peak demands.

When a tissue becomes starved of oxygen and its local P_{O2} falls below 40 mm Hg, the hemoglobin flowing through that tissue is on the steep portion of its sigmoid binding curve. That means that relatively small decreases in P_{O2} below 40 mm Hg will result in the release of lots of O₂ to the tissue. Thus the cooperativity of O₂ binding by hemoglobin is very effective in making O₂ available to the tissues precisely when and where it is needed most.

Myoglobin holds an oxygen reserve

Muscle cells have their own oxygen-binding molecule, myoglobin. Myoglobin consists of just one polypeptide chain associated with an iron-containing ring structure that can bind one molecule of oxygen. Myoglobin has a higher affinity for O₂ than hemoglobin does (see Figure 48.15), so it picks up and holds oxygen at P_{O2} values at which hemoglobin is releasing its bound O₂.

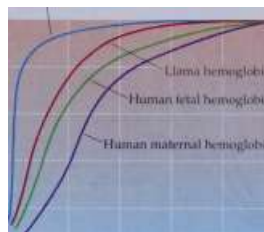
Myoglobin provides a reserve of oxygen for the muscle cells for times when metabolic demands are high and blood flow is interrupted. Interruption of blood flow in muscles is common because contracting muscles constrict blood vessels. When tissue P_{O2} values are low and hemoglobin can no longer supply more O₂, myoglobin releases its bound O₂. Diving mammals such as seals have high concentrations of myoglobin in their muscles, which is one reason they can stay underwater for so long. (We will learn more about adaptations for diving in the next chapter.) Even in nondiving animals, muscles called on for extended periods of work frequently have more myoglobin than muscles that

GAS EXCHANGE IN ANIMALS 861

Myoglobin

100
80 -
60 -
60
60
40
20 -

Llama hemoglobin Human fetal hemoglobin



Human maternal hemoglobin

20
40
60
80
100
< ^
P_{O2} (mm Hg)
T



48.15 Oxygen-Binding Adaptations

Evolution has adapted the oxygen-binding properties of different hemoglobins and of myoglobin. The hemoglobin of llamas, for example, is adapted for binding oxygen at high altitudes, where P_{O2} is low.

are used for short, intermittent periods. This is one of the reasons for the difference in appearance between fast-twitch and slow-twitch muscle (see Figure 47.12).

The affinity of hemoglobin for oxygen is variable

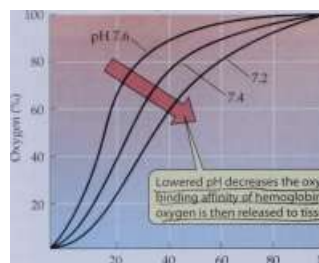
Various factors influence the oxygen-binding properties of hemoglobin, thereby influencing oxygen delivery to tissues. In this section we examine three of these factors: the chemical composition of the hemoglobin, pH, and the presence of 2,3 diphosphoglyceric acid.

hemoglobin composition. As we noted above, there is more than one type of hemoglobin. The chemical composition of the polypeptide chains that form the hemoglobin molecule varies. The normal hemoglobin of adult humans has two each of two kinds of polypeptide chains—two alpha chains and two beta chains—and the oxygen-binding characteristics shown in Figure 48.14.

Before birth, the human fetus has a different form of hemoglobin, consisting of two alpha chains and two gamma chains. The functional difference between these two types of hemoglobin is that the fetal hemoglobin has a higher affinity for O_2 . Therefore, the hemoglobin- O_2 binding curve of fetal hemoglobin is shifted to the left in comparison to the curve for adult hemoglobin (Figure 48.15). You can see from these curves that if both types of hemoglobin are at the same P_{O_2} , the fetal hemoglobin will pick up oxygen released by the adult hemoglobin. This difference in O_2 affinities facilitates the transfer of O_2 from the mother's blood to the blood of the fetus in the placenta.

Llamas and vicunas are mammals native to high altitudes in the Andes Mountains of South America. In the natural habitat of these animals, more than 5,000 m above sea

862 CHAPTER FORTY-EIGHT



Lowered pH decreases the oxygen-binding affinity of hemoglobin; more oxygen is then released to tissues.

40 60

P_{O_2} (mm Hg)

100

48.16 The Oxygen-Binding Properties of Hemoglobin Can Change

Changes in pH affect the oxygen-binding capacity of hemoglobin.



level, the P_{O_2} is below 85 mm Hg, and the P_{O_2} in their lungs is about 50 mm Hg. Thus, the hemoglobins of these animals must be able to pick up O_2 in an environment that has a low P_{O_2} . The hemoglobins of llamas and vicunas have oxygen-binding curves to the left of hemoglobins of most other mammals—in other words, their hemoglobin can become saturated with O_2 at lower P_{O_2} values than those of other animals can.

pH. The oxygen-binding properties of hemoglobin are influenced by physiological conditions. The influence of pH on the function of hemoglobin is known as the Bohr effect. As the blood plasma picks up acidic metabolites such as lactic acid, fatty acids, and CO_2 (which combines with water to form carbonic acid) from the tissues, its pH falls. When this happens, the oxygen-binding curve of hemoglobin shifts to the right (Figure 48.16). This shift means that the hemoglobin will release more O_2 to the tissues—another way that O_2 is supplied where and when it is most needed.

2,3 diphosphoglyceric acid. 2,3 diphosphoglyceric acid (DPG) is a metabolite in the glycolytic pathway. Mammalian red blood cells have a high concentration of DPG, an important regulator of hemoglobin function. DPG⁻, reversibly combines with deoxygenated hemoglobin and ν^{\wedge} changes the shape of the hemoglobin such that it has a lower affinity for O_2 . The result is that at any P_{O_2} , hemoglobin releases more of its bound O_2 than it otherwise would. In other words, DPG shifts the oxygen-binding curve of mammalian hemoglobin to the right. When humans go to high altitudes, or when they cease being sedentary and begin to exercise, the level of DPG in their red blood cells goes up and makes it easier for the hemoglobin to deliver more O_2 to the tissues. The reason that fetal hemoglobin has a left-shifted hemoglobin- O_2 binding curve is that it has a lower affinity for DPG than does adult hemoglobin.

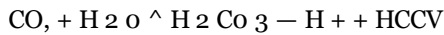
Llamas and humans employ opposite adjustments of hemoglobin function as adaptations for life at high altitudes. The llama's hemoglobin has a left-shifted oxygen-binding curve, which means that it can become 100 percent saturated with O_2 at the low P_{O_2} values at high altitudes. As a consequence, the llama's tissues must operate at a lower P_{O_2} . By contrast, human hemoglobin achieves, through acclimation, a right-shifted oxygen-binding curve. The result is that human

hemoglobin never becomes fully saturated with O_2 at high altitudes, but more of the O_2 carried by that hemoglobin is released to the tissues.

Carbon dioxide is transported as bicarbonate ions in the blood

Delivering O_2 to the tissues is only half of the respiratory function of the blood. The blood also must take carbon dioxide, a metabolic waste product, away from the tissues. CO_2 is highly soluble and readily diffuses through cell membranes, moving from its site of production in a cell into the blood, where the partial pressure of CO_2 is lower. However, very little dissolved CO_2 is transported by the blood. Most CO_2 produced by the tissues is transported to the lungs in the form of the bicarbonate ion HCO_3^- . How and where CO_2 is converted to HCO_3^- , is transported, and then is converted back to CO_2 is an interesting story.

When CO_2 dissolves in water, some of it slowly reacts with the water molecules to form carbonic acid (H_2CO_3), some of which then dissociates into a proton (H^+) and a bicarbonate ion (HCO_3^-). This reversible reaction is expressed as follows:



In the blood plasma, the reaction between CO_2 and H_2O proceeds slowly. But it is a different story in the endothelial cells of the capillaries and the red blood cells, where the enzyme carbonic anhydrase speeds up the conversion of CO_2 to H_2CO_3 . The newly formed carbonic acid dissociates and the resulting bicarbonate ions enter the plasma in exchange for Cl^- (Figure 48.17). By converting CO_2 to H_2CO_3 , carbonic anhydrase reduces the partial

pressure of CO_2 in these cells and in the plasma, facilitating the diffusion of CO_2 from tissue cells to endothelial cells, plasma, and red blood cells. Most CO_2 is transported by the blood plasma as bicarbonate ion produced in endothelial cells and red blood cells. Some CO_2 is also carried in chemical combination with deoxygenated hemoglobin as carboxyhemoglobin.

In the lungs, the reactions involving CO_2 and bicarbonate ions are reversed. CO_2 diffuses from the pulmonary capillaries to the alveolar air and is exhaled. Since the partial pressure of CO_2 in the alveoli is less than the partial pressure in the plasma and in the endothelial cells, CO_2 leaves the plasma and the endothelial cells and enters the alveoli. As the partial pressure in the plasma falls, CO_2 diffuses from the red blood cells into the plasma and from there into the endothelial cells and into the alveoli. As the partial pressure of CO_2 in the red blood cells falls, more HCO_3^- is converted into CO_2 , and more HCO_3^- moves into the red blood



figure 48.17

GAS EXCHANGE IN ANIMALS 863

In body tissues,

CO_2 diffuses from cells into plasma and into the red blood cells.

Some CO_2 combines with hemoglobin (Hb).

In the red blood cells, and in the endothelium, CO_2 is rapidly converted to bicarbonate ions because carbonic anhydrase is present.

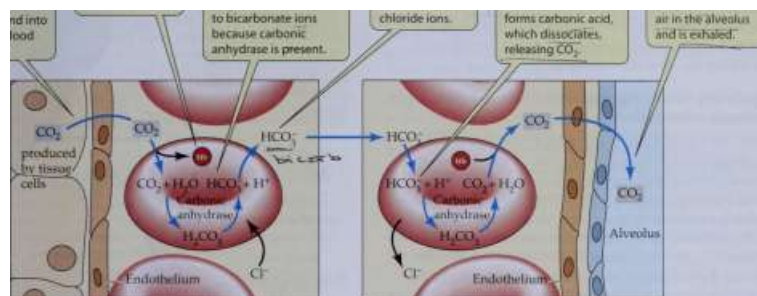
Bicarbonate ions enter the plasma in exchange for chloride ions.

In the lungs

these processes are reversed. Bicarbonate forms carbonic acid, which dissociates, releasing CO_2 .

CO_2 diffuses out of the RBC to the blood plasma and to the air in the alveolus

and is exhaled



Q. —Endotheli

Body tissue

Blood capillary

Blood capillary

Lung

48.17 Carbon Dioxide Is Transported as Bicarbonate Ions

Carbonic anhydrase in capillary endothelial cells and in red blood cells facilitates conversion of CO_2 produced by tissues into bicarbonate ions, carried by the plasma. In the lungs, the process is reversed

as CO_2 is exhaled.

cells from the plasma. Remember that an enzyme like carbonic anhydrase only speeds up a reversible reaction; it does not determine its direction. Direction is determined by concentrations of reactants and products (see Chapter 6).

Regulating Breathing to Supply O_2

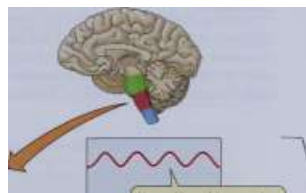
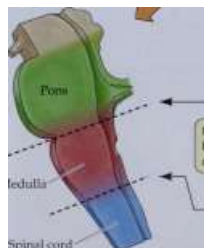
We must breathe every minute of our lives, but we don't worry about our need to breathe, or even think about it very often. Breathing is an autonomic function of the nervous system. The breathing pattern easily adjusts itself around other activities (such as speech and eating), and breathing rates change to match the metabolic demands of our bodies. In this section we examine how the regular breathing cycle is generated and controlled.

Breathing is controlled in the brain stem

The autonomic nervous system maintains breathing and modifies its depth and frequency to meet the demands of the body for O_2 supply and CO_2 elimination. Breathing ceases if the spinal cord is severed in the neck region, showing that the breathing pattern is generated in the brain. If the brain stem is cut just above the medulla, the segment of the brain stem just above the spinal cord, an irregular breathing pattern remains (Figure 48.18).

Groups of neurons within the medulla increase their firing rates just before an inhalation begins. As more and more of these neurons fire—and fire faster and faster—the diaphragm contracts. Suddenly the neurons stop firing, the diaphragm relaxes, and exhalation begins. Exhalation is

usually a passive process that depends on the elastic recoil of the lung tissues. When breathing demand is high, however, as during strenuous exercise, not only are the intercostal muscles recruited, which increases both the inhalation and the exhalation volumes. Brain areas above the medulla modify breathing to accommodate speech, ingestion of food, coughing, and emotional states.



Normal breathing requires an intact brain stem.

•n/W\w\

If the brain stem is cut below the pons but above the medulla, breathing continues but is irregular.

Mr

Medulla

Spinal cord

If the spinal cord in the neck is severed, breathing ceases.



48.18 The Brain Stem Generates and Controls Breathing Rhythm

Severing the brain stem at different levels reveals that the basic breathing rhythm is generated in the medulla and modified by neurons in or above the pons.

864 CHAPTER FORTY-EIGHT

An override reflex prevents the breathing muscles from overdistending and damaging the lung tissue. This reflex, which is called the Hering-Breuer reflex, begins with stretch receptors in the lung tissue. When stretched, these receptors send impulses to the medulla that inhibit the inhalation neurons.

Regulating breathing requires feedback information

When the P_{O_2} and the P_{CO_2} in the blood change, the breathing rhythm changes to return these values to normal levels. We should therefore expect the blood partial pressure of one or both of these gases to provide feedback information to the breathing rhythm generator. Experiments in which subjects breathe gases with different P_{O_2} and P_{CO_2} make it possible to measure the effect of these changes on breathing (Figure 48.19). In these experiments, it is assumed that the P_{O_2} or P_{CO_2} in inhaled air will be reflected in the blood. The conclusion from such experiments is that humans and other mammals are remarkably insensitive to falling blood levels of O_2 , but very sensitive to increases in the CO_2 of the blood.

Where are gas partial pressures in the blood sensed? The major site of CO_2 sensitivity is an area on the ven-

tral surface of the medulla, not far from the groups of neurons that generate the breathing rhythm. Sensitivity to O_2 in the blood resides in nodes of tissue on the large blood vessels leaving the heart: the aorta and the carotid arteries (Figure 48.20). These carotid and aortic bodies receive enormous supplies of blood, and they contain chemoreceptor nerve endings. If the blood supply to these structures decreases, or if the P_{O_2} of the blood falls dramatically, the chemoreceptors are activated and send impulses to the breathing control center. Although we are not very

Percent CO_2 in inhaled air (■ 2 3 4 5 6 7

10

-P 80

1

•—

01

r, |

C 60 -

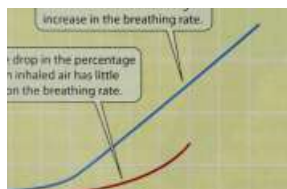
- 40

CQ

20 -

A small amount of CO_2 in inhaled air stimulates a large increase in the breathing rate.

A large drop in the percentage of O_2 in inhaled air has little effect on the breathing rate.



18 16 14 12 10 8 6 4 2 0 Percent O_2 in inhaled air ()

48.19 Carbon Dioxide Affects Breathing Rate

Breathing is more sensitive to increased carbon dioxide content in inhaled air than to decreased oxygen content.

From higher brain centers

Chemoreceptors on the surface of the medulla are sensitive to the partial pressure of carbon dioxide in the blood.

Chemoreceptors on large blood vessels leaving the heart are sensitive to the oxygen in the blood.

Blood to head

Carotid body

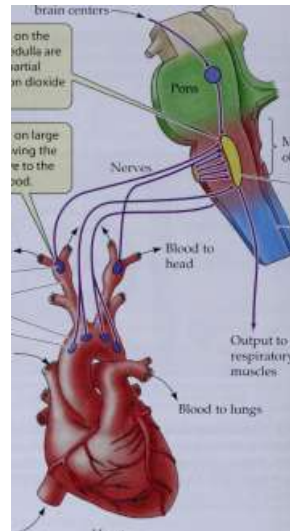
Carotid artery

Aorta

Aortic body

Blood from head

Blood from body



„ Medulla oblongata

Respiratory control area

Vt- Spinal cord

Output to

respiratory

muscles

Blood to lungs

Heart

48.20 Feedback Information Controls Breathing

The body uses feedback information from chemosensors in the heart and the brain to match breathing rate to metabolic demand.

sensitive to changes in blood P_{O_2} , the carotid and aortic bodies can stimulate increases in breathing during exposure to very high altitudes or when blood volume or blood pressure is very low.

rn



Chapter Summary

Respiratory Gas Exchange

► Most cells require a constant supply of O_2 and continuous removal of CO_2 . These respiratory gases are exchanged between the body fluids of an animal and its environment by diffusion.

► In aquatic animals, gas exchange is limited by the low diffusion rate and low amount of oxygen in water. Aquatic animals face a double bind in that the amount of oxygen in water decreases, but their metabolism and the amount of work required to move water over gas exchange surfaces increase, as water temperature rises. Review Figure 48.2

Respiratory Adaptations for Gas Exchange

► The evolution of large animals with high metabolic rates required the evolution of adaptations to maximize the rates of diffusion of respiratory gases between animals and their environments. These adaptations involve increasing surface areas

for gas exchange and maximizing partial pressure gradients across those exchange surfaces by decreasing their thickness, ventilating the outer surface with respiratory

GAS EXCHANGE IN ANIMALS 865

medium, and perfusing the inner surface with blood. Review Figure 48.4

- Insects distribute air throughout their bodies in a system of tracheae, tracheoles, and air capillaries. Review Figure 48.5

- ▶ Fish have maximized their rates of gas exchange by having large gas exchange surface areas that are ventilated continuously and unidirectionally with fresh water. Countercurrent blood flow helps increase the efficiency of gas exchange. Review Figures 48.6, 48.7

- ▶ The gas exchange system of birds includes air sacs that communicate with the lungs but are not used for gas exchange. Air flows unidirectionally through bird lungs in parabronchi. Gases are exchanged in air capillaries that run between parabronchi. Review Figures 48.8, 48.9

- ▶ Each breath of air remains in the bird respiratory system for two breathing cycles. The air sacs work as bellows to supply the air capillaries with a continuous, unidirectional flow of fresh air. Review Figure 48.10

- ▶ Breathing in vertebrates other than birds is tidal and is therefore less efficient than gas exchange in fishes or birds. Even though the volume of air exchanged with each breath can vary considerably, the inhaled air is always mixed with stale air. Review Figure 48.11

Mammalian Lungs and Gas Exchange

- ▶ In mammalian lungs, the gas exchange surface area provided by the millions of alveoli is enormous, and the diffusion path length between the air and perfusing blood is very short. Review Figure 48.12

- ▶ Surface tension in the alveoli would make their inflation difficult if the lungs did not produce surfactant.

- ▶ Inhalation occurs when contractions of the diaphragm and the intercostal muscles create negative pressure in the thoracic cavity. Relaxation of the diaphragm and some intercostal muscles and contraction of other intercostal muscles increases pressure in the thoracic cavity and causes exhalation. Review Figure 48.13

Blood Transport of Respiratory Gases

- ▶ Oxygen is reversibly bound to hemoglobin in red blood cells. Each molecule of hemoglobin can carry a maximum of four molecules of oxygen. Because of positive cooperativity, the affinity of hemoglobin for oxygen depends on the P_{O_2} to which the hemoglobin is exposed. Therefore, hemoglobin gives up oxygen in metabolically active tissues and picks up oxygen as it flows through respiratory exchange structures. Review Figure 48.14

- ▶ Myoglobin has a very high affinity for oxygen and serves as an oxygen reserve in muscle.
- ▶ There is more than one type of hemoglobin. Fetal hemoglobin has a higher affinity for oxygen than does maternal hemoglobin, allowing fetal blood to pick up oxygen from the maternal blood in the placenta. Review Figure 48.15
- ▶ The affinity of hemoglobin for oxygen is decreased by the presence of hydrogen ions or 2,3 diphosphoglyceric acid. Review Figure 48.16
- ▶ Carbon dioxide is carried in the blood principally as bicarbonate ions. Review Figure 48.17

Regulating Breathing to Supply O_2

- ▶ The breathing rhythm is an autonomic function generated by neurons in the medulla of the brain stem and modulated by higher brain centers. Review Figure 48.18
- ▶ The most important feedback stimulus for breathing is the level of CO_2 in the blood. Review Figure 48.19
- The breathing rhythm is sensitive to feedback from chemoreceptors on the ventral surface of the brain stem and in the carotid and aortic bodies on the large vessels leaving the heart. Review Figure 48.20

For Discussion

1. A species of fish that lives in Antarctica has no hemoglobin. What anatomical and behavioral characteristics would you expect to find in this fish, and why is its distribution limited to the waters of Antarctica?
2. Blood banks store whole blood for a much shorter period than they store blood plasma. The reason is that when blood that has been stored for too long is infused into a patient, it can actually decrease the oxygen availability to the patient's tissues. Why is this so? Explain in terms of the different physiological functions of 2,3 diphosphoglyceric acid.
3. Explain how llamas and humans can have opposite adaptations for maximizing gas transport at high altitudes.
4. In the disease emphysema, the fine structures of alveoli break down, resulting in the formation of larger air cavities in the lungs. Also, the tissue of the lungs becomes fibrotic and less elastic. Explain at least two reasons why patients with emphysema have a low tolerance for exercise.
5. The disease called "the bends" occurs in scuba divers (persons who spend time underwater by breathing pressurized air) who have come too quickly to the surface after spending an extended period in deep water. The cause of the bends is tiny bubbles of nitrogen coming out of solution in the blood plasma. Seals spend much more time underwater and at deeper depths than scuba divers, yet they do not suffer the bends. Why?



Circulatory Systems



Your heart is a little bigger than

your fist. This mass of muscle pumps about 200 ml of blood to your lungs and an equal amount to the rest of the organs of your body with each beat, and when you are at rest, it beats about once each second. Even when you are at rest, your heart pumps your total blood volume through your lungs and around your body about once each minute.

Circulating blood has many functions, such as delivery of oxygen and nutrients to cells, removal of waste products of metabolism, and distribution of heat and hormones. It is not surprising, therefore, that when you work or exercise, your heart rate and the amount of blood your heart pumps

each minute go up as much as three or four times to match the increasing metabolic demands. Because blood flow can be redistributed to different tissues and organs depending on their needs, blood flow to your active muscles might increase more than 25-fold during exercise.

It makes sense that working muscles should get a greater blood supply. Consider, however, the cardiovascular responses of a seal searching for and pursuing prey underwater for over half an hour. The seal's response to this underwater exercise is very different from yours. Its heart slows dramatically, from about 150 beats per minute to 20, its cardiac output falls proportionally, and blood flow to the muscles propelling its swimming falls practically to zero. Blood flow to the heart is less than a third what it was before the dive began. Only blood flow to its nervous system is maintained at pre-dive levels. Because the circulatory system of the seal responds differently during exercise than yours does, the seal is able to conserve its oxygen supplies and remain underwater for long periods. By the end of this chapter, you will understand the adaptations of circulatory systems that enable you to match blood supply with demand in your exercising muscles and enable the seal to decrease its utilization of oxygen during long dives.

We begin this chapter by contrasting the open and closed circulatory systems of invertebrates. Then we discuss the evolution, structure, and function of vertebrate circulatory systems, going

Champion Divers

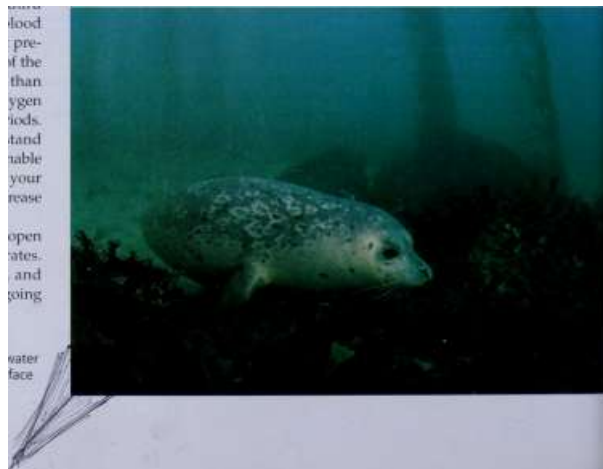
A harbor seal (*Phoca vitulina*) can be active underwater for 30 minutes or more without coming to the surface to breathe.

from the two-chambered hearts and single blood circuits of fishes to the four-chambered hearts and double blood circuits of birds and mammals. Taking the human heart as a model, we explore the mechanics of the beating heart and the events of the cardiac cycle that pump blood around the body.

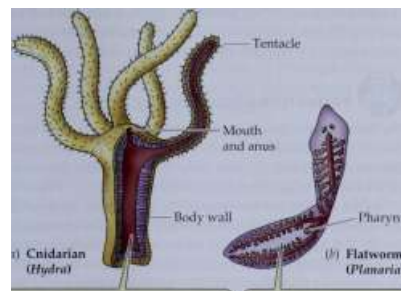
After the heart, we turn to the characteristics of the vascular system: the arteries, capillaries, and veins. We explain how materials are exchanged between the blood and the tissue fluids. The third component of a circulatory system is the blood, and we describe the features of this fluid tissue. The chapter ends with a discussion of the hormonal and neural control and regulation of the human circulatory system and an explanation of the diving adaptations of marine mammals.

Circulatory Systems: Pumps, Vessels, and Blood

A circulatory system consists of a pump (heart), a fluid (blood) that can transport materials, and a series of connected blood vessels through which the fluid can be pumped around the body. Heart, blood, and vessels are also known as a cardiovascular system (from the Greek *kardia*, "heart," and the Latin *vascidum*, "small vessel"). In this sec-



Tentacle



(a) Cnidarian (Hydra)

Pharynx

The gastrovascular cavity of a flatworm, *Planaria*, extends into all regions of the animal's flattened body.

The gastrovascular cavity of a cnidarian, *Hydra*, extends into the tentacles. No cell of the hydra is more than one cell away from either the gastrovascular cavity or the external medium.

49.1 Gastrovascular Cavities

In small aquatic animals without circulatory systems, a gastrovascular cavity serves the metabolic needs of the innermost cells of the body.

tion, we compare the circulatory systems of different groups of animals.

Some simple aquatic animals do not have circulatory systems

A circulatory system is unnecessary if the cells of an organism are close enough to the external environment that nutrients, respiratory gases, and wastes can diffuse between the cells and the environment. Small aquatic invertebrates have structures and body shapes that permit direct exchanges -between cells and environment. The hydra, a cnidarian, is a good example (see Figure 42.1a). All cells of the hydra are in contact with, or very close to, the water that either surrounds the

animal or circulates through its gastrovascular cavity, a dead-end sac that serves both for digestion ("gastro-") and for transport ("vascular") (Figure 49.1a). The cells of some other invertebrates are served by highly branched gastrovascular systems, and many have flattened body shapes that maximize the surface area of the animal that is in contact with the external environment (Figure 49.1b). •

Large surface-to-volume ratios and branched gastrovascular systems cannot satisfy the needs of larger animals with many layers of cells. The cells of these animals are surrounded by internal, but extracellular, fluids—commonly called tissue fluids. Circulatory systems carry materials to and from all regions of the body to maintain the optimum composition of the tissue fluids, which in turn serve the needs of the cells. Terrestrial animals require circulatory systems because none of their cells are bathed by an external aqueous medium, and all their cells must be served by tissue fluids.

Open circulatory systems move tissue fluid

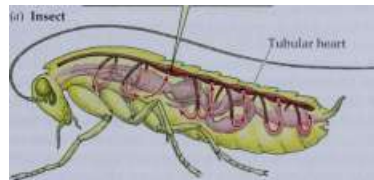
The simplest circulatory systems squeeze tissue fluid through intercellular spaces and the animal moves. In these open circulatory systems, there is no distinction between tissue fluid and blood. Usually a muscular pump, or heart, assists the distribution of the fluid. The contractions of the heart propel the tissue fluid through vessels leading to different regions of the body, but the fluid leaves those vessels to trickle through the tissues and eventually return to the heart. In the arthropod shown in Figure 49.2a, the fluid returns to the heart through y-shaped holes called ostia. In the mollusk in Figure 49.2b, open vessels aid in the return of tissue fluid to the heart.

Closed circulatory systems circulate blood through tissues

In a closed circulatory system, a system of vessels keeps circulating blood separate from the tissue fluid. Blood is pumped through this vascular system by one or more muscular hearts, and some components of the blood never leave the vessels.

In arthropods, tissue fluid reenters the heart through the ostia.

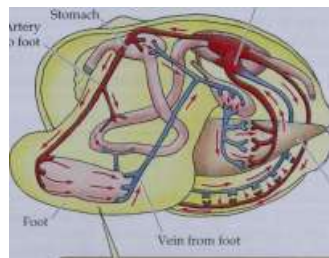
Insect



(b) Mollusk

Stomach Artery to foot

Heart



Gills

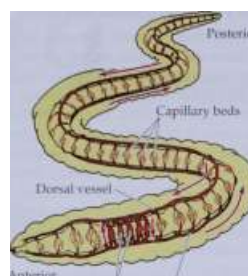
Vein from foot

In mollusks a system of vessels drains the intercellular spaces and returns the blood to the heart.

49.2 Open Circulatory Systems

In both arthropods (a) and mollusks (b), blood is pumped by a tubular heart and directed to regions of the body through vessels that open into intercellular spaces.

868 CHAPTER FORTY-NINE



Anterior

Hearts

Ventral vessel

49.3 A Closed Circulatory System

In a closed circulatory system, blood is confined to the blood vessels, kept separate from the tissue fluid, and pumped by one or more muscular hearts. The earthworm, with large dorsal and ventral blood vessels and a branching network of smaller vessels, exemplifies this type of system.

A simple example of a closed circulatory system is that of the earthworm/an annelid (see Figure 31.23). One large blood vessel on the ventral side of the earthworm carries blood from its anterior end to its posterior end. Smaller vessels branch off and transport the blood to even smaller vessels serving the tissues in each segment of the worm's body. In the smallest vessels, respiratory gases, nutrients, and metabolic wastes diffuse between the blood and the tissue fluid. The blood then flows from these vessels into larger vessels that lead into one large vessel on the dorsal side of the worm. The dorsal vessel carries the blood from the posterior to the anterior end of the body. Five pairs of vessels connect the large dorsal and ventral vessels in the anterior end, thus completing the circuit (Figure 49.3). The dorsal vessel and the five connecting vessels serve as hearts for the earthworm; their contractions keep the blood circulating. The direction of circulation is determined by one-way valves in the dorsal and connecting vessels.

Closed circulatory systems have several advantages over open systems!

- ▶ Blood can flow more rapidly through vessels than through intercellular spaces, and therefore can transport nutrients and wastes to and from tissues more rapidly.
- ▶ By changing resistances in the vessels, closed systems can selectively direct blood to specific tissues.
- ▶ Cellular elements and large molecules that aid in the transport of hormones and nutrients can be kept within the vessels.

Overall, closed circulatory systems can support higher levels of metabolic activity, especially in larger animals. How, then, do highly active insect species achieve high levels of metabolic output with their open cir-



culatory systems? One answer to this question can be found in Chapter 48: Insects do not depend on their circulatory systems for respiratory gas exchange (see Figure 48.5).

Vertebrate Circulatory Systems

Vertebrates have closed circulatory systems and hearts with two or more chambers. Valves between the chambers, and between the chambers and the vessels, prevent the back-flow of blood when the heart contracts.

As we explore the features of the circulatory systems of the different classes of vertebrates, a general evolutionary theme will become apparent: there is a progressively more complete separation of the circulation of blood to the gas exchange organ from the circulation of blood to the rest of the body. In fishes, blood is pumped from the heart to the gills and then to the tissues of the body and back to the heart. In birds and mammals, blood is pumped from the heart to the lungs and back to the heart in a pulmonary circuit, and then from the heart to the rest of the body and back to the heart in a systemic circuit. We will trace the evolution of the separation of the circulation into two circuits.

The closed vascular system of vertebrates includes arteries that carry blood away from the heart and veins that carry blood back to the heart. Arterioles are small arteries, and venules are small veins. Capillaries are tiny, thin-walled vessels that permit the exchange of materials between the blood and the tissue fluid only across capillary walls.

Fishes have two-chambered hearts

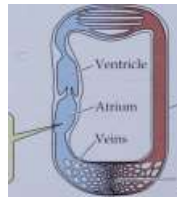
The fish heart has two chambers. A less muscular chamber, called the atrium, receives blood from the body and pumps it into a more muscular chamber, the ventricle. The ventricle pumps the blood to the gills, where gases are exchanged. Blood leaving the gills collects in a large dorsal artery, the aorta, which distributes blood to smaller arteries and arterioles leading to all the organs and tissues of the body. In the tissues, blood flows through beds of tiny capillaries, collects in venules and veins, and eventually returns to the heart.

Gills

■ Oxygenated blood

1 Deoxygenated blood

Fishes have a heart with two chambers: a single atrium and a single ventricle.



Aorta

Systemic capillaries

Most of the pressure imparted to the blood by the contraction of the ventricle is dissipated by the high resistance of the narrow spaces through which blood flows in the

CIRCULATORY SYSTEMS 869

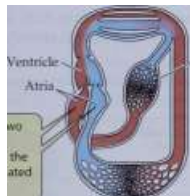
gills. As a result, blood entering the aorta of the fish is under low pressure, limiting the capacity of the fish circulatory system to supply the tissues with oxygen and nutrients. This limitation on arterial blood pressure does not seem to hamper the performance of many rapidly swimming species, such as tuna and marlin.

The evolutionary transition from breathing water to breathing air had important consequences for the vertebrate circulatory system. An example of how the system changed to serve a primitive lung is seen the African lung-fish. These fish are periodically exposed to water with low oxygen content or to situations in which their aquatic environment dries up. Their adaptation for dealing with these conditions is an outpocketing of the gut that serves as a lung. The lung contains many thin-walled blood vessels, so blood flowing through those vessels can pick up oxygen from air gulped into the lung.

How does the circulatory system take advantage of this new organ? The last pair of gill arteries is modified to carry blood to the lung, and a new vessel carries oxygenated blood from the lung back to the heart. In addition, two other gill arches have lost their gills, and their blood vessels deliver blood from the heart directly to the dorsal aorta. Because a few of the gill arches retain gills, the African lung-fish can breathe either air or water.

Gills

The lungfish heart has two atria, one receiving oxygenated blood from the lung and one deoxygenated blood from the body.



Lung

The lungfish heart has adaptations that partially separate the flow of its blood into pulmonary and systemic circuits. Unlike other fishes, the lungfish has a partly divided atrium; the left side receives oxygenated blood from the lungs, and the right side receives deoxygenated blood from the other tissues. These two bloodstreams stay mostly separate as they flow through the ventricle and the large vessel leading to the gill arches, so that the oxygenated blood goes to the gill arteries leading to the dorsal aorta, and the deoxygenated blood goes to the arches with functional gills and to the lung.

Amphibians have three-chambered hearts

Pulmonary and systemic circulation are partly separated in adult amphibians. A single ventricle pumps blood to the lungs and to the rest of the body. Two atria receive blood returning to the heart. One receives oxygenated blood from the lungs, and the other receives deoxygenated blood from the body.

Because both atria deliver blood to the same ventricle, the oxygenated and deoxygenated blood could mix so that blood going to the tissues would not carry a full load of oxygen. Mixing is limited, however, because anatomical features of the ventricle direct the flow of deoxygenated blood from the right atrium to the pulmonary circuit and the flow of oxygenated blood from the left atrium to the aorta.

Lung

■ Oxygenated TM blood

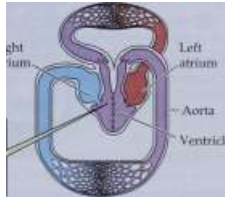
~| Deoxygenated LJ blood

j Mixed blood

Right atrium

In adult amphibians, the

pulmonary and systemic circuits are partially separated. The heart has three chambers.



The advantage of this partial separation of pulmonary and systemic circulation is that the high resistance of the gas exchange organ no longer lies between the heart and the tissues. Therefore, the amphibian heart delivers blood to the aorta, and hence to the body, at a higher pressure than the fish heart does.

Reptiles have exquisite control of pulmonary and systemic circulation

Turtles, snakes, and lizards are commonly said to have three-chambered hearts, while crocodilians (crocodiles and alligators) are said to have four-chambered hearts. But this statement is an oversimplification. The hearts of all these animals have two separate atria and a ventricle that is divided in a complex way so that mixing of oxygenated and deoxygenated blood is minimized.

The most important and unusual feature of reptilian and crocodilian hearts is their ability to alter the distribution of blood going to the lungs and to the rest of the body. Consider the behavior, ecology, and physiology of these animals. Despite the common image of turtles as being slow and plodding, reptiles and crocodilians can be fast, active, powerful animals. They can also be inactive for long periods of time, during which they have metabolic rates much lower than the resting metabolic rates of birds and mammals. The enormous range of metabolic demands in these animals means that they do not have to breathe continuously. Some species are also accomplished divers and spend long periods underwater where they cannot breathe.

To understand the wonderful adaptations of the reptilian and crocodilian hearts, you have to realize that there is no benefit in sending blood to the lungs when an animal is not breathing. The hearts of these animals circulate blood through their lungs and then to the rest of their bodies when they are breathing, but when they are not breathing, they can bypass the lung circuit and pump all the blood around the body. How do they accomplish this switching?

870 CHAPTER FORTY-NINE

Reptiles have two aortas instead of one. This simplified representation of reptilian cardiovascular anatomy shows that the right aorta can receive blood from either the right side or the left side of the ventricle:

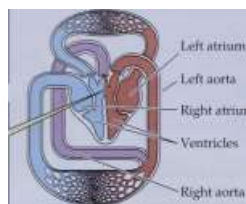
■ Oxygenated blood

~| Deoxygenated blood

I Mixed blood

Lung

The reptilian ventricle is partly divided by a septum to direct oxygenated blood to the body and deoxygenated blood to the lungs.



Right aorta

When the animal is breathing air, two factors cause blood from the right side of the ventricle to go preferentially into the pulmonary circuit rather than into the systemic circuit. First, the resistance in the pulmonary circuit is lower than that in the systemic circuit. Second, there is a slight asynchrony in the timing of ventricular contraction, so the blood in the right side of the ventricle tends to be ejected slightly before the blood in the left side. As the ventricle contracts, the deoxygenated blood in the right side of the ventricle moves first into the lung circuit. When the oxygenated blood in the left side of the ventricle starts to move, it encounters resistance in the lung circuit, which is already filled with the deoxygenated blood from the right side. Therefore the blood from the left side tends to flow into the two aortas.

When the reptile stops breathing, blood flow is rerouted by constriction of vessels in the lung circuit. As resistance in the lung circuit increases, the blood from the right side of the ventricle tends to be directed into one of the aortas. As a result, blood from both sides of the ventricle flows through the aortas to the systemic circuit.

The ability of snakes, lizards, and turtles to redirect blood flow from the lung circuit to the systemic circuit depends on the incomplete division of their ventricles. Crocodilians have true four-chambered hearts with completely divided ventricles. Yet the crocodilians have not lost the ability to shunt blood from the lung circuit when they are not breathing. The crocodilians have one aorta originating in the left ventricle and one aorta originating in the right ventricle. However, a short

channel connects these two aortas just after they leave the heart.

Because the crocodilians' ventricles are separate, they can generate different pressures when they contract. When the animal is breathing, the pressure in the left ventricle and the left aorta is higher than the pressure in the right ventricle. This higher pressure is communicated through the connecting channel to the right aorta, and this high back pressure prevents right-ventricle blood from entering that aorta. As a result, both aortas carry blood from the left ventricle, and the blood from the right ventricle flows to the lung circuit.

■ Oxygenated blood

I Deoxygenated L i blood

J Mixed blood

Lung

Crocodilians have completely divided ventricles, but they still have the ability to alter the distribution of blood to the pulmonary and systemic circuits because of a connection between two of the major vessels leaving the heart.



Aorta from right ventricle

Aorta from left ventricle

When a crocodilian is not breathing, constriction of vessels in the lung circuit increases the resistance in that circuit. As a result, pressure builds up in the right ventricle to a level that exceeds the back pressure in the right aorta. Under these conditions, blood from both ventricles flows through the two aortas and the systemic circuit, and little blood flows into the lung circuit.

You can now appreciate the fact that reptilian and crocodilian hearts are not primitive. Rather, these hearts and their major vessels are highly adapted to operate efficiently over a wide range of metabolic demands.

Birds and mammals have fully separated pulmonary and systemic circuits

The four-chambered hearts of birds and mammals completely separate their pulmonary and systemic circuits.

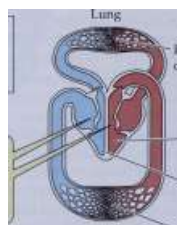
■ Oxygenated ** blood

"1 Deoxygenated L —' blood

Birds and mammals

have four-chambered hearts. Their pulmonary and systemic circuits are totally separate.

Separate circuits have several_adv's



Pulmonary circuit

Left ventricle

Right ventricle

Systemic

► Oxygenated and deoxygenated blood cannot mix, and therefore the systemic circuit is always receiving blood with the highest oxygen content.

► Respiratory gas exchange is maximized because the blood with the lowest oxygen content and highest CO₂ content is sent

to the lungs.

► Separate systemic and pulmonary circuits can operate at different pressures.

The tissues of birds and mammals have high nutrient demands and thus a very high density of the smallest vessels, the capillaries. Many small vessels present lots of resistance to the flow of blood. Therefore, high pressure is re-

quired in the systemic circuits of birds and mammals. Their pulmonary circuits do not have as many capillaries and as high resistances as their systemic circuits, so the pulmonary circuits of birds and mammals can run at lower pressures. „ ^ _ -

The Human Heart: Two Pumps in One

Like all other mammalian hearts, the human heart has four chambers: two atria and two ventricles (Figure 49.4). The atrium and ventricle on the right side of your body are called the right atrium and right ventricle. They can be thought of as the right heart. The atrium and ventricle on the left side of your body are called the left atrium and left ventricle. They can be thought of as the left heart. The right heart pumps blood through the pulmonary circuit, and the left heart pumps blood through the systemic circuit. ^

valves between the atria and ventricles, the atrioventricular valves, prevent backflow of blood into the atria when the ventricles contract. The pulmonary valve and the aortic valve are situated between the ventricles and the arteries, ^^^^AP prevent the backflow of blood into the ventricles.

In what follows, we'll first focus on the flow of blood through the heart and through the body. Then we'll exam-

N-J

&

-O

<?ine the unique electrical properties of cardiac muscle and see how the heart's electrical activity can be recorded in an ^EKG (electrocardiogram).

,>r

- - Blood flows from right heart to lungs

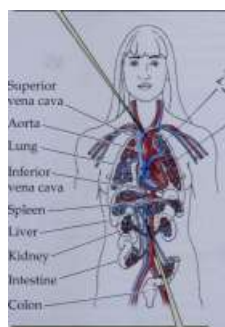
.-js&Vpttffeft heart to body

r>

xP

Let's follow the circulation of the blood through the heart, starting in the right heart. The right atrium receives deoxygenated blood from the superior vena cava and the infe-

Vessels colored in red bring oxygenated blood from the lungs to the left heart, which pumps it to the rest of the body.



Pulmonary valve

Superior vena cava

4

From lung

Q Deoxygenated blood from the tissues of the body enters the right atrium.

Q ... and flows through an atrioventricular valve into the right ventricle.

Vessels shown in blue bring deoxygenated blood from the body to the right heart, which pumps it to the lungs for oxygenation.

§JThe right ventricle pumps the blood into the pulmonary circuit.

rior vena cava (see Figure 49.4), large veins that collect blood from the upper and lower body, respectively. The veins of the heart itself also drain into the right atrium. From the right atrium, the blood flows into the right ventricle. Most of the filling of the ventricle is due to passive flow while the heart is relaxed between beats. Just at the end of this period of ventricular filling, the atrium contracts and adds a little more blood to the ventricular volume. The right ventricle then contracts, pumping blood into the pul-

monarily~artery, which transports the blood

g-

The pulmonary veins lead oxygenated blood from the lungs to the left atrium, from which the blood enters the left ventricle. As with the right side of the heart, most left ventricular filling is passive, and ventricular volume is topped off by contraction of the atrium just at the end of the period of filling.

The walls of the left ventricle are powerful muscles that contract around the top with a wringing motion starting from the bottom. When pressure in the left ventricle is high enough to push open the aortic valve, the blood rushes into the aorta to begin its circulation throughout the body and eventually back to the right atrium. In Figure 49.4, observe that the left ventricle is more massive than the right ventricle. Because there are many more arterioles and capillaries in the systemic circuit than in the pulmonary circuit, resistance is higher in the systemic circuit, and the left ventricle must squeeze with greater force than the right, even though both are pumping the same volume of blood.

Figure 49.4 The Human Heart and Circulation

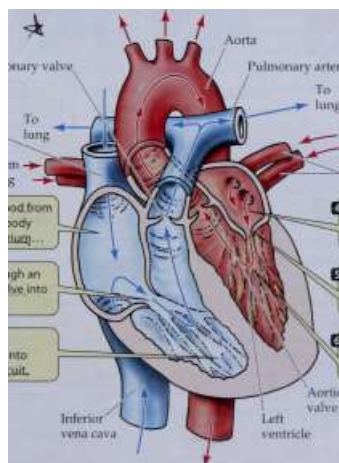
In the human heart, blood flows from right heart to lungs to left heart to body. The atrioventricular valves prevent blood from flowing back into the atria when the ventricles contract. The pulmonary and aortic valves prevent blood from flowing back into ventricles from the arteries when the ventricles relax.



Aorta

Pulmonary artery

To lung



From lung

Pulmonary veins

From the pulmonary circuit, the blood returns to the left atrium...

... and flows through an atrioventricular valve into the ventricle.

The left ventricle pumps blood into the systemic circuit.

Inferior vena cava

Aortic

valve Left

ventricle

The pumping of the heart—the contraction of the two atria followed by the contraction of the two ventricles, and then relaxation—is the cardiac cycle. Contraction of the ventricles is called systole, and relaxation of the ventricles is diastole (Figure 49.5). Just at the end of diastole, the atria contract and top off the volume of blood in the ventricles. The sounds of the cardiac cycle, the "lub-dub" heard through a stethoscope placed on the chest, are created by the slamming shut of the heart valves. The shutting and opening of these valves is simply a mechanical event resulting from pressure differences on the two sides of the valves. As the ventricles begin to contract, the pressure in the ventricles rises above the pressure in the atria, and the atrioventricular valves close ("lub"). When the ventricles begin to relax, the high back pressure in the aorta and pulmonary arteries causes the aortic and pulmonary valves to bang shut ("dub"). Defective valves produce the sounds of heart murmurs. For example, if an atrioventricular valve is defective, blood will flow back into the atrium with a "whoosh" sound following the "lub."

The cardiac cycle can be felt in the pulsation of arteries such as the one that supplies blood to your hand. You can feel your pulse by placing two fingers from one hand

49.5 The Cardiac Cycle

The rhythmic contraction (systole) and relaxation (diastole) of the ventricles is called the cardiac cycle.



lightly over the wrist of the other hand just below the thumb. During systole, blood surges through the arteries of your arm and hand, and you can feel the surge as a pulsing of the artery in your wrist.

Blood pressure changes associated with the cardiac cycle can be measured in the large artery in your arm by using an inflatable pressure cuff called a sphygmomanometer and a stethoscope (Figure 49.6). This method measures the minimum pressure necessary to compress an artery so that blood does not flow through it at all (the systolic value) and the minimum pressure that permits intermittent flow through the artery (the diastolic value). In a conventional blood pressure reading, the systolic value is placed over the diastolic value. Normal values for a young adult might be 120 mm of mercury (Hg) during systole and 80 mm Hg during diastole, or 120/80.

The heartbeat originates in the cardiac muscle

Cardiac muscle, as we saw in Chapter 47, has some unique properties that allow it to function as an effective pump. First, the cardiac muscle cells are in electrical continuity with each other. Gap junctions enable action potentials to spread rapidly from one cell to the next. Because a spreading action potential stimulates contraction, large groups of cardiac muscle cells contract in unison. This coordinated contraction is important for pumping blood.

"Lub": The ventricles contract, the atrioventricular valve shuts, and pressure in the ventricles builds up until the aortic and pulmonary valves open.

"Dub": The ventricles relax; pressure in the ventricles falls at the end of systole, and since pressure is higher in the aorta, the aortic and pulmonary valves slam shut.

Left atrium

Right atrium

Right ventricle

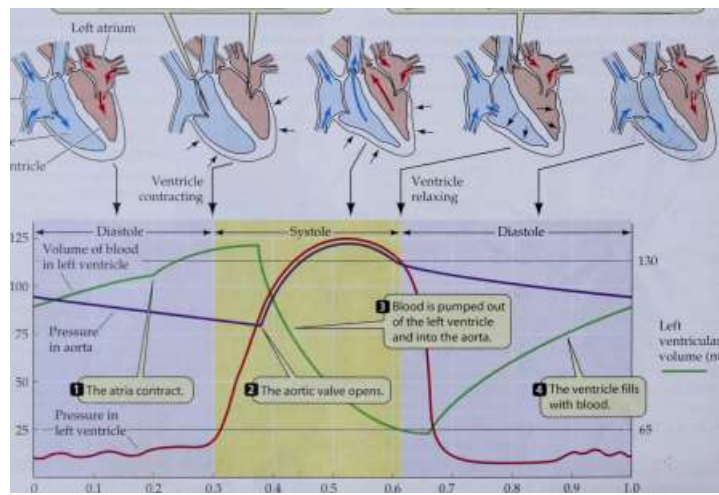
Left ventricle

Pressure in

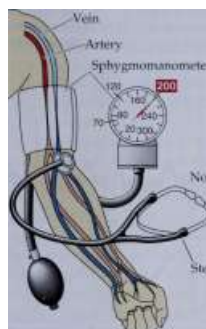
left ventricle

and aorta

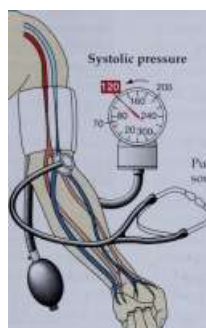
(mmHg)



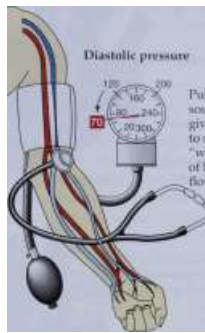
Left
ventricular volume (ml)
0.4 0.5 0.6
Time (sec)
(«)
-Artery Sphygmomanometer



(6)
Systolic pressure
200
No sounds
Stethoscope



(c)
Diastolic pressure
Pulsing sounds



Pulsing sound gives way to smooth "whoosh" of blood flow

Pressure in the cuff is increased to close both the arteries and veins. No sound is audible.

Pressure in the cuff is gradually lowered until the sound of a pulsing flow of blood through the constriction in the artery during systole is heard. At this time, pressure in the cuff is just below the peak systolic pressure in the artery.

Pressure is further lowered until the sound becomes continuous when the artery remains open for an entire cardiac cycle. The cuff is just below the diastolic pressure in the artery at this time. Blood pressure in this person is 120/70.

49.6 Measuring Blood Pressure

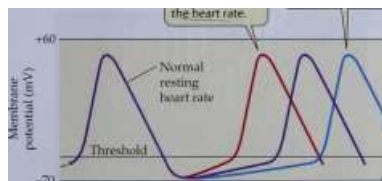
Blood pressure in the major artery of the arm can be measured with an inflatable pressure cuff called a sphygmomanometer.

Second, some cardiac muscle cells have the ability to initiate action potentials

system. These cells stimulate neighboring cells to contract, thereby acting as pacemakers. The important characteristic of a pacemaker cell is that its resting membrane potential gradually becomes less negative until it reaches the threshold voltage for initiating an action potential (Figure 49.7).

Sympathetic autonomic activity increases the heart rate.

Parasympathetic autonomic activity slows the heart rate.



Time

49.7 The Autonomic Nervous System Controls Heart Rate

The resting potentials of the pacemaker cells spontaneously generate action

potentials. Signals from the two divisions of the autonomic nervous system raise and lower the heart rate, respectively.

These action potentials look different from the neuronal action potentials you saw in Chapter 41 because the depolarization is due primarily to the opening of voltage-gated calcium channels rather than voltage-gated sodium channels.

Like neurons, cardiac muscle regularizes in part by opening potassium channels. The potassium channels in cardiac pacemaker cells, however, are unique. After an action potential, they open, causing the membrane potential to fall to its most negative level. Then they gradually close, and as they do so, the membrane potential becomes less negative—it slowly depolarizes. Sodium and calcium channels contribute to this gradual depolarization between action potentials. When membrane potential reaches threshold for the voltage-gated calcium channels, another action potential occurs.

The nervous system controls the heartbeat (speeds it up or slows it down) by influencing the rate at which pacemaker cells gradually depolarize between action potentials. Acetylcholine released by parasympathetic nerve endings onto the pacemaker cells slows their rate of depolarization and thereby

lowers heart rate. No

by sympathetic nerve endings onto the pacemaker cells increases their rate of depolarization and thereby speeds the heart rate (see Figure 49.7).

Under normal circumstances, the heartbeat originates from pacemaker cells located at the junction of the superior vena cava and right atrium, in the sinoatrial node (Figure 49.8). An action potential spreads from the sinoatrial node across the atrial walls, causing the two atria to contract in unison. Since there are no gap junctions between the atria and the ventricles, however, this depolarization does not

QRS

QRS

"

QRS

Besides detecting rhythmic irregularities in the heartbeat (arrhythmias), EKGs can detect damage to the heart muscle (infarctions) or decreased blood supply to the heart muscle (ischemias) by changes in the size and shape of the EKG curves.

The action potential initiated in the atria passes to the ventricles through another node of modified cardiac muscle cells, the atrioventricular node. The atrioventricular node passes the action potential on to the ventricles via modified muscle fibers called the bundle of His. The bundle of His divides into right and left branches, which connect with Purkinje fibers that branch throughout the ventricular muscle.

The timing of the spread of the action potential from atria to ventricles is important. The atrioventricular node imposes a short delay in the spread of the action potential from atria to ventricles. Then the action potential spreads very rapidly throughout the ventricles, causing them to contract. Thus the atria contract before the ventricles do, so the blood passes progressively from the atria to the ventricles to the arteries.

(c)



49.9 The Electrocardiogram

An EKG can be used to monitor heart function.

The EKG records the electrical activity of the heart

Electrical events in the cardiac muscle during the cardiac cycle can be recorded by electrodes placed on the surface of the body. Such a recording is called an electrocardiogram, or EKG ("EKG" because the Greek word for heart is kardia, but "ECG" is also used). The EKG is an important tool for diagnosing heart problems.

The action potentials that sweep through the muscles of the atria and the ventricles before they contract are such massive, localized electrical events that they cause electric currents to flow outward from the heart to all parts of the body. Electrodes placed on the surface of the body at different locations—usually on the wrists and ankles—detect those electric currents at different times and therefore register a voltage difference. The appearance of the EKG depends on the exact placement of the electrodes used for the recording. Electrodes placed on the right wrist and left ankle produced the normal EKG shown in Figure 49.9a. The waves of the EKG are designated P, Q, R, S, and T, each letter representing a particular event in the cardiac muscle.

The EKG is used by cardiologists (heart specialists) to diagnose heart problems. Figure 49.9b shows some abnormal EKGs that result from different problems. For patients who have had heart attacks, it is possible to determine which region of the heart has been damaged by placing electrodes at

different locations on the chest. Comparing EKGs from the different electrodes tells the cardiologist which region of the heart is behaving abnormally.

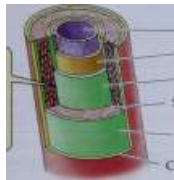
The Vascular System: Arteries, Capillaries, and Veins

Blood circulates throughout the body in a system of blood vessels: arteries, capillaries, and veins. Arteries receive blood from the heart; accordingly, they withstand high pressures. Arteries are important in controlling blood pressure and in the distribution of blood to different organs. Veins, return blood to the heart at low pressures and serve as blood reservoir. Capillaries are the site of all exchanges between the blood and the internal environment. In this section, we see how the structure of each of these vessel types supports its functions. In addition to arteries, capillaries, and veins, we consider another set of vessels, the lymphatic vessels, which return tissue fluid to the blood.

Arteries and arterioles have abundant elastic and muscle fibers

The walls of the large arteries have many elastic fibers that enable them to withstand high pressures (Figure 49.10). These elastic fibers have another important function as

Arteries have lots of elastic fibers and muscle fibers, allowing them to withstand high pressures.

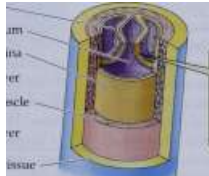


Valve Endothelium Basal lamina Elastic layer

Smooth muscle

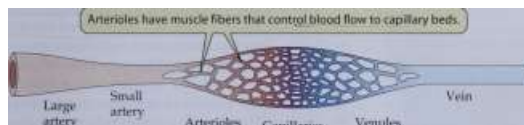
Elastic layer

Connective tissue



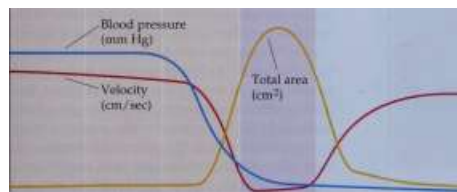
Because veins operate under low pressure, some veins have valves to prevent backflow of blood.

Arterioles have muscle fibers that control blood flow to capillary beds.



Large arteries

Arterioles Capillaries Venules



Small arteries

Arterioles Capillaries Venules

Veins

, A^{***}

[r7!^ 49.10 Anatomy of Blood Vessels

The anatomical characteristics of blood vessels match their functions. The total cross-sectional area of capillaries is greater than that of any other class of vessels, and they are more permeable, a characteristic that suits them for their function of exchange of nutrients and wastes with the tissue fluids.



876 CHAPTER FORTY-NINE

well: During systole, they are stretched, and thereby store some of the energy imparted to the blood by the heart. During diastole, they return this energy by squeezing the blood and pushing it forward. As a result, even though the flow of blood through the arterial system pulsates, it is smoother than it would be through a system of rigid pipes.

Smooth muscle cells in the arteries and arterioles make the diameter of these vessels variable. When their diameter changes, their resistance to blood flow changes as well, and the amount of blood flowing through them changes as a result. By influencing the contraction of the smooth muscle in the walls of arteries and arterioles, neural and hormonal mechanisms can control the distribution of blood to the different tissues of the body as well as central blood pressure. The arteries and arterioles are referred to as the resistance vessels because their resistance varies.

Materials are exchanged between blood and tissue fluid in the capillaries

Beds of capillaries lie between arterioles and venules. No cell of the body is more than a couple of cell diameters away from a

capillary. The needs of the cells are served by the exchange of materials between blood and tissue fluid across the capillary walls. Capillaries have thin, permeable walls, and blood flows through them slowly, facilitating this exchange (Figure 49.11).

To anyone who has played with a garden hose, it may seem strange that blood flows through the large arteries rapidly at high pressures, but when it reaches the small capillaries, the pressure and rate of flow decrease. When you restrict the diameter of a garden hose by placing your thumb over the opening, the pressure in the hose increases, which in turn

increases the velocity of the water spraying out of the hose.

This puzzle is solved by two more pieces of information.

First, arterioles are highly branched. When one is restricted, blood flows into other branches, so pressure does not build

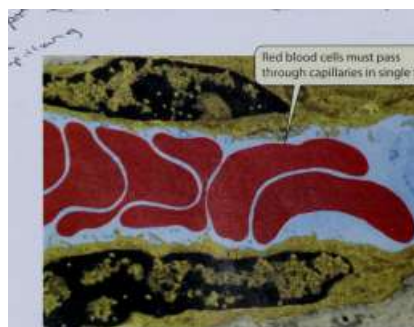
,

f

,

V

Red blood cells must pass through capillaries in single file.



49.7 A Narrow Lane

Capillaries have a very small diameter, and blood flows through them slowly.

f

up quickly. Second, each arteriole gives rise to many, many

capillaries. Even though each capillary has a diameter so small that red blood cells must pass through in single file (see Figure 49.11), there are so many capillaries that their total cross-sectional area is much greater than that of any other class of vessel. As a result, all of the capillaries together have a much greater capacity for blood than do the arterioles. Returning to our garden hose analogy, if we connect the hose to many junctions leading to small irrigation tubes, the pressure and the flow in each of the irrigation tubes will be quite low.

Materials are exchanged in capillary beds by filtration, osmosis, and diffusion

The walls of capillaries are made of a single layer of thin endothelial cells. In most tissues of the body other than the brain, these tubes of endothelial cells have tiny holes called fenestrations. Surrounding the endothelial cells is a very permeable basal lamina. So, capillaries are leaky. They are permeable to water, to some ions, and to some small molecules, but not to large molecules such as proteins. Blood pressure therefore tends to squeeze water and some solutes out of the capillaries, and into the surrounding intercellular spaces. This process is called filtration. The large molecules that cannot cross the capillary wall create a difference in osmotic potential (also called osmotic pressure) between the plasma and the tissue fluid, which tends to draw water back into the capillary.

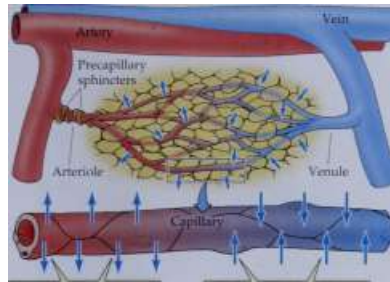
Recent research suggests that bicarbonate ions in the capillary plasma are an important contributor to the osmotic attraction of water back into the capillary. As we saw in Chapter 48, CO_2 diffuses into the plasma as the blood flows through the capillary. The conversion of this CO_2 to

bicarbonate ions is catalyzed by the enzyme carbonic anhydrase. Therefore, there is a rise in bicarbonate concentration as blood flows through the capillary, and this increased concentration contributes to the resorption of water from the tissue fluid.

Blood pressure is highest on the arterial side of a capillary bed and steadily decreases as the blood flows to the venous side. Therefore, more water is squeezed out of the capillaries on the arterial side of the bed. The osmotic potential is pulling water back into the capillary rises as blood flows toward the venous side. Gradually, osmotic potential becomes dominant.

The dominant force, pulling water back into the plasma. The interaction of these two opposing forces— blood pressure and osmotic potential—

π versus osmotic potential—determines the net flow of water $\Delta \pi$ between the plasma and the tissue fluid (Figure 49.12). The balance between blood pressure and osmotic potential changes if the blood pressure in the arterioles or the permeability of the capillary walls changes. Such a change leads to the inflammation that accompanies injuries to the skin or allergic reactions. A major mediator of inflammation is a hormone called histamine that is released mainly by white blood cells, called mast cells, that move to the damaged tissue (see Chapter 41). Histamine relaxes the smooth muscle of the arterioles, thus increasing blood flow to the damaged tissue and increasing pressure in the capillaries.



Fluid is squeezed out of the capillary by blood pressure.

Fluid is pulled back into the capillary by osmotic potential.

50

r 23

Xet driving force for fluid to leave capillar}

Net driving force for fluid to reenter capillary



Osmotic potential

Blood pressure

Arteriole end

mm H^g Hydrostatic pressure 40 Osmotic potential

-20

Xet outward force

20

Venule end

mm Hs Hydrostatic pressure 16 Osmotic potential -30

Net inward force

14

49.12 A Balance of Opposite Forces

Blood pressure and osmotic attraction (both expressed as millimeters of mercury, mm HG) control the exchange of fluids between blood vessels and intercellular space.

Histamine also increases the permeability of the capillaries,

CIRCULATORY SYSTEMS 877

The capillaries in different tissues, however, are differentially selective as to the sizes of molecules that can pass through them. In all auxiliaries. $Q : \text{CO}^+$, glucose, lactate, and small ions such as Na^+ and Cl^- can cross. The capillaries of the brain do not have fenestrations, and therefore not much else can pass through them unless it is a lipid-soluble substance such as alcohol. This high selectivity of brain capillaries is known as the blood-brain barrier (see Chapter 44).

In other tissues, the capillaries are much less selective. Such capillaries are found in the digestive tract, where nutrients are absorbed, and in the kidneys, where wastes are filtered. Some capillaries have large gaps that permit the movement of even larger substances, such as red blood cells. These capillaries are found in the bone marrow, spleen, and liver. Substances move across many capillary walls by endocytosis (see Chapter 5).

Lymphatic vessels return tissue fluid to the blood

The tissue fluid that accumulates outside the capillaries contains water and small molecules, but no red blood cells, and less protein than there is in blood. A separate system of vessels—the lymphatic system—returns tissue fluid to the blood.

After entering the lymphatic vessels, the tissue fluid is called lymph. Fine lymphatic capillaries merge progressively into larger and larger vessels and end in a major vessel—the thoracic duct—that empties into the superior vena cava, returning blood to the heart (see Figure 19.1). Lymphatic vessels have one-way valves that keep the lymph flowing toward the thoracic duct. The force propelling the lymph is pressure on the lymphatic vessels from the contractions of nearby skeletal muscles.

Mammals and birds have lymph nodes along the major lymphatic vessels. Lymph nodes are an important component of the defensive machinery of the body (see Chapter 19). They are a major site of lymphocyte production and of

so that more water leaves the vessels. The accumulation of the phagocytic action that removes microorganisms and

fluid in the intercellular spaces causes the tissue to swell, a condition known as edema. The use of drugs called antihistamines can alleviate inflammation and allergic reactions.

The loss of water from capillaries increases if the protein content of the blood decreases, as is seen in cases of liver failure due to alcoholism or liver disease. The liver is the major producer of blood proteins. If it fails, blood protein levels fall. With a lower protein concentration in the plasma, there is less of an osmotic potential to pull water back into the capillaries. The result is that tissue fluid builds up, swelling the abdomen and the extremities.

Which specific small molecules can cross a capillary wall depends on the architecture of the capillary, the type of substance, and the concentration gradient of the substance between the blood and the tissue fluid. Capillary walls consist of the plasma membranes of endothelial cells and, as mentioned, may have actual holes (fenestrations) in them. Therefore, lipid-soluble substances and many small solute molecules can pass through them from an area of higher concentration to one of lower concentration (see Chapter 5).

Other foreign materials from the circulation. The lymph nodes also act as mechanical filters. Particles become trapped there and are digested by the phagocytes that are abundant in the nodes.

Lymph nodes swell during infection. Some of them, particularly those on the sides of the neck or in the armpits, become noticeable when they swell. The nodes also trap metastasized cancer cells—that is, those that have broken free of the original tumor. Because such cells may start additional tumors, surgeons often remove the neighboring lymph nodes when they excise a malignant tumor.

Blood flows back to the heart through veins

The pressure of the blood flowing from capillaries to venules is extremely low, and is insufficient to propel blood back to the heart. Blood tends to accumulate in veins, and the walls of veins are more expandable than the walls of arteries. As much as 80 percent of the total blood volume may be in the veins at any one time. Because of their high capacity to store blood, veins are sometimes called blood reservoirs.

878 CHAPTER FORTY-NINE

Blood must be returned from the veins to the heart so that circulation can continue. If the veins are above the level of the heart, gravity helps blood flow, but below the level of the heart, gravity.

If too much blood remains in the veins, then too little blood returns to the heart, and thus too little blood is pumped to the brain; a person may faint as a result. Fainting is self-correcting: if a person falls, thereby moving out of the position in which gravity caused blood to accumulate in the lower body. But means other than fainting also move blood from the tissues back to the heart.

The most important of the forces that propel venous and lymphatic return from the regions of the body below the heart is the squeezing of the vessels by the contractions of skeletal muscles. As muscles contract, the vessels are compressed, and the blood is squeezed through them. Blood flow might be temporarily obstructed during a

■ Δ



a***



prolonged muscle contraction, but with relaxation of the muscles the blood is free to move again.

One-way valves within the veins prevent the backflow of blood. Thus whenever a vein is squeezed, blood is propelled forward toward the heart (Figure 49.13). As we have already noted, the lymphatic vessels have similar one-way valves.

Gravity causes blood accumulation in veins and edema. The back pressure that builds up in the capillaries when blood

accumulates in the veins shifts the balance between blood pressure and osmotic potential so that there is a net movement of fluid into the intercellular spaces. That is why you have trouble putting your shoes back on after you sit for a long time with your shoes off, such as on an airline flight. In persons with very expandable veins, the veins can become so stretched that the valves can no longer prevent backflow. This condition produces (microscopic) (swollen) veins. Draining of these veins is highly desirable and can be aided by wearing support hose and periodically elevating the legs above the level of the heart.

During exercise, the squeezing action of muscles on veins speeds blood toward the heart to be pumped to the lungs and then to the respiring tissues. As an animal runs, its legs act as auxiliary vascular pumps, returning blood to the heart from the veins of the lower body. As a greater volume of blood is returned to the heart, the heart contracts more forcefully, and its pumping action becomes more effective. This strengthening of the heartbeat is due to a property of cardiac muscle cells referred to as the Frank-Starling law: If the cells are stretched, as they are when the volume of returning blood increases, they contract more forcefully. This principle holds (within a certain range) whenever venous return increases, by any mechanism.

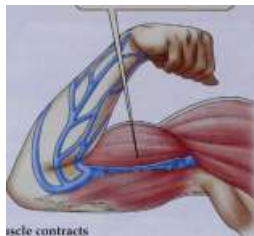
The actions of breathing also help return venous blood to the heart. The ventilatory muscles create suction that pulls air into the lungs (see Chapter 48), and this suction also pulls blood and lymph toward the chest, increasing venous return to the right atrium.

Some smooth muscles in the walls of veins move venous blood back to the heart by constricting the veins and moving

or

or?

Contractions of skeletal muscles squeeze the veins.



Muscle contracts

Valve closed 111 Valve open

\\



This squeezing moves the blood in the veins toward the heart because of one-way valves that prevent backflow.

Muscle relaxes

Valve open

Valve closed



Blood is propelled forward by muscle contractions and, possibly, by gravity.

Back pressure is due to contractions of atria, contractions of muscles, and, possibly, gravity.

49.73 One-Way Flow

Veins have valves that prevent blood from flowing backward.

ing the blood forward. These muscles are rare in most veins and are totally absent from lymphatic vessels in humans. They do not play a major role in venous return. However, in the largest veins closest to the heart, contraction of smooth muscles at the onset of exercise can suddenly increase venous return and stimulate the heart in accord with the Frank-Starling law, thus increasing cardiac output.

Will you die of cardiovascular disease?

Cardiovascular disease is by far the largest single killer in the United States and Europe; it is responsible for about half of all deaths each year. The immediate cause of most of these deaths is heart attack or stroke, but those events are the end result of a disease called atherosclerosis (hardening of the arteries) that begins many years before symptoms are detected. Hence atherosclerosis is called the "silent killer." What is atherosclerosis, and how can it be prevented?

Healthy arteries have a smooth internal lining of endothelial cells (Figure 49. 14a). This lining can be damaged by chronic high blood pressure, smoking, a high-fat diet or microorganisms. Deposits called atheromas begin to form at sites of endothelial damage. First the endothelial cells at the damaged site swell and proliferate; then they are joined by smooth muscle cells migrating from below. Lipids, espe-

■ * ^

^^..p.

4<=

■ ^-t

^

(fl)



Thrombus

(b)



daily cholesterol, are deposited in these cells, so the plaque becomes fatty. Fibrous connective tissue invades the plaque and, along with deposits of calcium, makes the artery wall less elastic; this process is what gives us the terms "atherosclerosis" and "hardening of the arteries." The growing plaque narrows the artery and causes turbulence in the blood flowing over it. Blood platelets (discussed later in this chapter) stick to the plaque and initiate the formation of a blood clot, called a thrombus, which further blocks the artery (Figure 49. 14b).

The blood supply to the heart itself flows through the coronary arteries. These arteries are highly susceptible to atherosclerosis; as they narrow, blood flow to the heart muscles decreases. Chest pains and shortness of breath during mild exertion are symptoms of this condition. A person with atherosclerosis is at high risk of forming a thrombus in a coronary artery. This condition, called coronary thrombosis, can totally block the vessel, causing a heart attack, or coronary infarction.

~~. A piece of a thrombus that breaks loose, called an embolus,

is likely to travel to and become lodged in a vessel of

smaller diameter, blocking its flow (a condition referred to

as an embolism). Arteries already narrowed by plaque for-

CIRCULATORY SYSTEMS 879

49.74 Atherosclerotic Plaque

(a) A healthy, clear artery. (b) An atherosclerotic artery, clogged with plaque and a thrombus.

,<S^S- ^*" Q>

&*.*

V-tT ^

mation are likely places for an embolus to lodge. An embolism in an artery in the brain causes the cells fed by that artery to die. This event is called a stroke. The specific damage resulting from a stroke, such as memory loss, speech impairment, or paralysis, depends on the location of the blocked artery.

The most important approach to cardiovascular disease is prevention, not treatment. Probably the most important determinant of whether or not you will get atherosclerosis is your genetic predisposition. Environmental risk factors also play a large role, however, and if you do have a genetic predisposition to atherosclerosis, it is even more important to minimize environmental risk factors. These factors include high-fat and high-cholesterol diets, smoking, a sedentary lifestyle, hypertension (high blood pressure), obesity,

and certain medical conditions such as diabetes. Changes in diet and behavior can prevent and reverse atherosclerosis and help fend off the silent killer.

Blood: A Fluid Tissue

Blood is classified as a connective tissue: it has cellular ele-

ments suspended in an extracellular matrix of complex, yet specific, composition. The unusual feature of blood is that the extracellular matrix is a liquid, so blood is a fluid tissue.

The cells of the blood can be separated from the fluid matrix, called plasma, by centrifugation (Figure 49.15). If a 100-ml sample of blood is spun in a centrifuge, all the cells move to the bottom of the tube, leaving the straw-colored, clear plasma on top. The packed volume, or hematocrit, is the percentage of the blood volume made up by cells. Normal hematocrit is about 38 percent for women and 46 percent for men, but these values can vary considerably. They are usually higher, for example, in people who live and work at high altitudes because the low oxygen concentrations at high altitudes stimulate the production of more red blood cells.

In this section, we consider two classes of cellular elements in blood: the red blood cells and the platelets, which are pinched-off fragments of cells. We have already studied the other important class of blood cells—white blood cells, or leucocytes—in Chapter 19. Finally, we take a closer look at the content of plasma.

Red blood cells transport respiratory gases

Most of the cells in the blood are erythrocytes, or red blood cells. Mature red blood cells are biconcave, flexible discs packed with hemoglobin. Their function is to transport the respiratory gases. Their shape gives them a large surface area for gas exchange, and their flexibility enables them to squeeze through narrow capillaries. There are about 5 to 6 million red blood cells per milliliter of blood.

CHAPTER FORTY-NINE



90

80-

70

60

50-

40-30-20-10-

Blood is withdrawn from the arm, placed in a test tube, and centrifuged.

Plasma portion

Transported by blood: Nutrients

(e.g., glucose, vitamins) Waste products of metabolism Respiratory gases (O_2 and CO_2) Hormones Heat

Cellular portion (hematocrit)

XJ

Components

Number per mm³ of blood

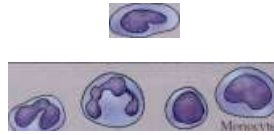
Functions

Erythrocytes (red blood cells)

Leukocytes (white blood cells)

5-6 million

Transport oxygen and carbon dioxide



Basophil

Eosinophil and Lymphocyte

Monocyte

5,000-10,000

Destroy foreign cells, produce antibodies; roles in allergic responses

Platelets



<Ss>

250,000-400,000

Blood

clotting

49.15 The Composition of Blood

Blood consists of a complex aqueous solution, numerous cell types, and cell fragments.

Red blood cells are generated by special cells in the bone marrow called stem cells, particularly in the bone marrow of the ribs, breastbone, pelvis, and vertebrae. Red blood cell production is controlled by a hormone, erythropoietin, which is released by cells in the kidney in response to insufficient oxygen (hypoxia) in the tissues (Figure 49.16a). Many tissues respond to hypoxia by expressing a transcription factor called hypoxia-inducible factor 1 (HIF-1). In the kidney, HIF-1 activates the gene encoding erythropoietin. Cells in the kidney produce erythropoietin, which stimulates the stem cells to produce red blood cells.

Under normal conditions, your bone marrow produces about 2 million red blood cells every second. The developing, immature red blood cells divide many times while still in the bone marrow, and during this time they produce hemoglobin. When the hemoglobin content of a red blood cell

approaches about 30 percent, its nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondria begin to break down. This process is almost complete when the new red blood cell squeezes between the endothelial cells of capillaries in the bone marrow and enters the circulation.

Each red blood cell circulates for about 120 days and then breaks down. As it gets older, its membrane becomes less flexible and more fragile. Therefore, old red blood cells frequently rupture as they bend to fit through narrow capillaries. One place where they are really squeezed is in the

spleen, an organ that sits near the stomach in the upper left side of the abdominal cavity. The spleen has many venous cavities, or sinuses, that serve as a reservoir for red blood cells, but to get into the sinuses, the red blood cells must squeeze between spleen cells. Old red blood cells are likely to be ruptured by this squeezing and when they are, their remnants are broken down by macrophages.

Platelets are essential for blood clotting

Besides producing red blood cells, the stem cells in the bone marrow produce the leukocytes and cells called megakaryocytes. Megakaryocytes are large cells that remain in the bone marrow and continually break off cell fragments called platelets (Figure 49.16/). A platelet is just a tiny fragment of a cell, but it is packed with enzymes and chemicals necessary for its function: sealing leaks in blood vessels and initiating blood clotting.

Damage to a blood vessel exposes collagen fibers. When a platelet encounters collagen fibers, it is activated. It swells, becomes irregularly shaped and sticky, and releases chemicals that activate other platelets and initiate the clotting of

bloodr The sticky platelets form a plug at the damaged site, and the subsequent clotting forms a stronger patch on the vessel.

~The~doTtmg of blood requires many steps and many clotting faejp rs. The absence oi any one o\ these factors can impair clotting and cause excessive bleeding. Because the lixejj^njduces most of the clotting factors, liver diseases such as he4^aiiis_ and cirrhosis can result in exce ssive

(a)

m



Kidney

produces

erythropoietin

Induction of HIF-1

Stimulates

Stem cells in bone marrow

If too low

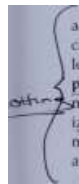
1

7

Produce

O, supply in tissues

Red blood cells



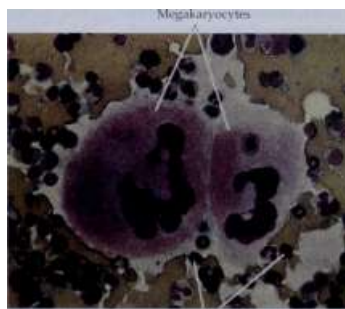
Increase

49.16 Formation of Red Blood Cells and Platelets

(a) Erythropoietin stimulates stem cells in the bone marrow to produce red blood cells, (b) In this micrograph, platelets can be seen breaking away from the edges of two megakaryocytes.

bleeding. The sex-linked trait hemophilia (see Chapter 10) is an example of a genetic inability tevp roduce one o f the clotting factors.

Blood clotting factors participate in a cascade of steps that activate other substances circulating in the blood. The cascade begins with cell damage and-plaJ£leLac±Lvation and leads to the con version of an inactive circulating enzyme, prothrombin, to its activ e form, thrombi n. Thrombin causes olecules of a plasma protein called fibrinogen to polymerize and form fibrin threads. The fibrin threads form the meshw ork that clo ts the blood, seals the vessel, and provides a scaffold for the formation of scar tissue (Figure 49.17).



Platelets

Plasma is a complex solution

Plasma, the clear straw-colored liquid portion of the blood, contains gases, ions, nutrient molecules, proteins, and other molecules, such as nonprotein hormones. Most of the ions are Na^+ and Cl^- (hence the salty taste of blood), but many other ions are also present. Nutrient molecules in plasma include glucose, amino acids, lipids, cholesterol, and lactic acid.

The circulating proteins in plasma have many functions. We have just noted proteins that function in blood clotting; others of interest include albumin, which is partly responsible for the osmotic potential in capillaries that prevents a massive loss of water from plasma to intercellular spaces; antibodies (the immunoglobulins); hormones; and various carrier molecules, such as transferrin, which carries iron from the gut to where it is stored or used.

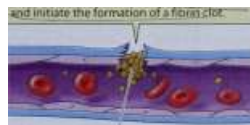
(f)

An injury to the lining of a blood vessel exposes collagen fibers; platelets adhere and become sticky.

Platelet



Platelets release substances that cause the vessel to contract. Sticky platelets form a plug and initiate the formation of a fibrin clot.



Red blood cell

Collagen fibers

Platelet plug

Clotting factors

1. Released from platelets and injured tissue
2. Plasma proteins synthesized in liver and circulated in inactive form

49.77 Blood Clotting

(a) Damage to a blood vessel initiates a cascade of events that produces a fibrin meshwork. (b) As the meshwork forms, red blood cells are enmeshed in the fibrin threads, forming a clot.

Prothrombin circulating in plasma

Thrombin



Fibrinogen circulating in plasma

The fibrin clot seals the wound until the vessel wall heals.



Fibrin meshwork

882 CHAPTER FORTY-NINE

Plasma is very similar to tissue fluid in composition, and most of its components move readily between these two fluid compartments of the body. The main difference between the two fluids is the higher concentration of proteins in the plasma.

Precapillary sphincters can shut off blood supply to the capillary bed.

Control and Regulation of Circulation

Arteriole - Capillary —

Muscle fibers (cells).

The circulatory system is controlled and regulated by neural and hormonal mechanisms at both the local and systemic levels. Every tissue requires an adequate supply of blood that is saturated with oxygen, carries essential nutrients, and is relatively free of waste products. The nervous system cannot monitor and control every capillary bed in the body. Instead, each tissue regulates its own blood flow through autoregulatory mechanisms that cause the arterioles to constrict or dilate.

The autoregulatory actions of every capillary bed in every tissue influence the pressure and composition of the arterial blood leaving the heart. If many arterioles suddenly dilate, for example, allowing blood to flow through many more capillary beds, arterial blood pressure falls. If all the newly filled capillary beds contribute metabolic waste products to the blood at one time, the concentration of wastes in the blood returning to the heart increases. Thus events in all the capillary beds throughout the body produce

the combined effects on arterial blood pressure and blood composition. The nervous and endocrine systems respond to these changes by changing the heart rate, and blood distribution to match the metabolic needs of the

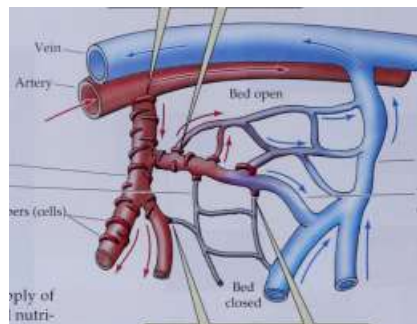
body.

Autoregulation matches local flow to local need

The autoregulatory mechanisms that adjust the flow of blood to a tissue are part of the tissue itself, but they can be influenced by the nervous system and certain hormones.

The amount of blood that flows through a capillary bed is controlled by the degree of contraction of the smooth muscle of the arteries and arterioles feeding that bed. The flow of blood in a typical capillary bed is diagrammed in Figure 49.18. Blood flows into the bed from an arteriole. Smooth muscle "cuffs," or precapillary sphincters, on the arteriole can completely shut off the supply of blood to the capillary bed. When the precapillary sphincters are relaxed and the arteriole is open, the arterial blood pressure pushes blood into the capillaries.

Autoregulation depends on the sensitivity of the smooth muscle to its chemical environment. Low O_2 concentrations and high CO_2 concentrations cause the smooth muscle to relax, thus increasing the supply of blood, which brings in more O_2 and carries away CO_2 . Increases in other by-products of metabolism, such as lactate, hydrogen ions, potassium, and adenosine, promote increased blood flow through



Venule

Throughfare channel

Blood flow through a capillary bed is controlled by the constriction of smooth muscle in the arteries and arterioles.

49.78 Local Control of Blood Flow

Low O_2 concentrations or high levels of metabolic by-products cause the smooth muscle of the arteries and arterioles to relax, thus increasing the supply of blood to the capillary bed.

the same mechanism. Hence, activities that increase the metabolism of a tissue also increase blood flow to that tissue.

Arterial pressure is controlled and regulated by hormonal and neural mechanisms

The same smooth muscle of arteries and arterioles that responds to autoregulatory stimuli also responds to signals from the endocrine and central nervous systems. Most arteries and arterioles are innervated by the autonomic nervous system, particularly the sympathetic division. Most sympathetic neurons release norepinephrine, which causes the smooth muscle cells to contract. This constricts the vessels and reduces blood flow. An exception is found in skeletal muscle, in which specialized sympathetic neurons release epinephrine, causing the smooth muscle of the arterioles to relax and the vessels to dilate, increasing blood flow to the muscle, especially during exercise.

Hormones also can cause arterioles to constrict or dilate.

Angiotensin II

Angiotensin II, which has actions similar to those of norepinephrine, is released from the adrenal medulla during exercise

sympathetic activation—the fight-or-flight response. Angiotensin, produced when blood pressure in the kidneys falls, causes arterioles to constrict. Vasopressin, released by the posterior pituitary when blood pressure falls, has similar effects (Figure 49.19). These hormones influence arterioles located for the most part in peripheral tissues (extremities) or in tissues whose functions need not be maintained continuously, (such as the gut). By reducing blood flow in those arterioles, the hormones increase central blood pressure and blood flow to essential organs such as the heart, brain, and kidneys.

•cr •

Hf Vill

Arterial pressure falls



CIRCULATORY SYSTEMS 883

c*-

Firing in

stretch sensors

decreases

Kidney releases

7

Decreased

blood flow to

tissue

Autoregulatory widening of vessels



Hypothalamus

releases

vasopressin

\

\

Local accumulation of metabolic wastes

Vasopressin causes vessels to

49.19 Hormonal Control of Blood Pressure through Vascular Resistance

A drop in arterial pressure reduces blood flow to tissues, resulting in local accumulation of metabolic wastes. This change in the extracellular environment stimulates autoregulatory opening of the arteries, which would lead to a further decrease in central blood pressure if not prevented by the negative feedback mechanisms shown in this diagram, which work by promoting the constriction of arteries in less essential tissues.

Arterial pressure rises

The autonomic nervous system activity that controls heart rate and constriction of blood vessels originates in cardiovascular centers in the medulla of the brain stem. Many inputs converge on this central integrative network and influence the commands it issues via parasympathetic and sympathetic nerves (Figure 49.20). Of special importance is information about changes in blood pressure from stretch receptors in the walls of the great arteries leading to the brain—the aorta and the carotid arteries.

^k

Increased activity in the stretch receptors indicates rising blood pressure and inhibits sympathetic nervous system output. As a result, the heart slows, and arterioles in peripheral tissues dilate. If pressure in the great arteries falls, the activity of the stretch receptors decreases, stimulating sympathetic output. As a result, the heart beats faster, and the arterioles in peripheral tissues constrict. When arterial pressure falls, the change in stretch receptor activity also causes the

hypothalamus to release vasopressin, which helps increase blood pressure by stimulating peripheral arterioles to constrict.

You experience the action of the aortic and carotid stretch receptors when you get up each morning. While you are lying down, your blood pressure is rather evenly distributed from head to toe, but when you get up gravity pulls blood to the lower part of your body. Blood return to the heart decreases, and therefore cardiac output decreases. As a result, the pressure in the aorta and the carotid arteries falls. This change is detected by the carotid and aortic stretch receptors, which initiate corrective responses within two heartbeats. Now imagine the change in blood pressure detected by the carotid stretch receptors in the giraffe in the cover of this book when it raises and lowers its head. The giraffe has a much larger heart than would be expected for a mammal of its size because of the need to generate blood pressure sufficient to pump blood against gravity to a height more than 3 m above its heart.



Higher brain centers

- Emotion
- Anticipation
- Stress

Chemoreceptors in medulla

/- ► IBP I* ->

Sympathetic II Parasympathetic

Kidney



Adrenal gland

o



Epinephrine I Increases @ f+) Decreases

heart rate j heart rate

Heart rate and arterial pressure

Stretch receptors in aorta and carotid artery



Chemoreceptors

on aorta and carotid arteries

49.20 Regulating Blood Pressure

The autonomic nervous system controls heart rate in response to information about blood pressure and blood composition that is integrated by regulatory centers in the medulla.

884 CHAPTER FORTY-NINE

Other information that causes the medullary regulatory system to increase heart rate and blood pressure comes from chemoreceptors in the carotid and aortic bodies. These nodules of modified smooth muscle tissue respond to inadequate O₂ supply. If arterial blood flow slows or the O₂ content of the arterial blood falls drastically, these receptors are activated and

send signals to the regulatory center. The regulatory center also receives input from other brain areas. Emotions or the anticipation of intense activity, as at the start of a race, can cause the center to increase heart rate and blood pressure.

Control and regulation in the cardiovascular system begins with the local autoregulatory mechanisms that cause dilation of local arterioles and precapillary sphincters when a tissue needs more oxygen or has accumulated wastes. As more blood flows into the tissues, the central blood pressure falls, and the composition of the blood returning to the heart reflects the exchanges that are occurring in the tissues. Changes in central blood pressure and blood composition are sensed, and both endocrine and central nervous system responses are activated to return blood pressure and composition to normal. Thus circulatory functions are matched to the regional and overall needs of the body.

Cardiovascular control in diving mammals conserves oxygen

We began this chapter with the observation that when a seal begins underwater activity, its heart rate slows and blood flow to all of its tissues except its brain drops dramatically. This "diving reflex" of marine mammals is in stark contrast to our Increase in heart rate and blood flow when we begin exercise. The obvious difference between the situation of the seal and the human is that the human has access to atmospheric oxygen during exercise and the diving seal does not.

The adaptations of the seal that enable it to remain underwater for a long time are several. The seal's oxygen storage capacity is about twice ours due to greater blood volume, the greater oxygen carrying capacity of its blood, and more myoglobin in its muscles. That is not sufficient, however, to explain dives of half an hour or more. The most important adaptation is the diving reflex, which is a slowing of the heart (Figure 49.21) and a constriction of major blood vessels going to all tissues except certain critical ones such as the nervous system, the heart, and the eyes. Central blood pressure remains high, but blood flow to the tissues decreases. This reduced blood flow has two effects: one is to switch the tissue to glycolytic (anaerobic) metabolism, and the other is to suppress the metabolism of the tissue.

While diving, the seal accumulates lactic acid in its muscles, which constitutes an "oxygen debt" to be paid back through elevated metabolism after the dive ends. But the total metabolic "payback" is much less than the metabolism that would have occurred over the same period of time had the seal not dived. The diving reflex caused the seal to be

Seal dives

0
180
160
140
120
100
80
60
40
20

Seal resurfaces

•%••.*..



......

Time (minutes)

49.21 The Diving Reflex

When a marine mammal dives, its heart rate slows and the arteries to most of its organs constrict, so almost all blood flow and available oxygen goes to the animal's heart and brain. These adaptations enable some seals to remain underwater for up to an hour.

hypometabolic (below the basal metabolic rate) during the dive. Hypometabolism, increased oxygen stores, and a high

capacity for anaerobic metabolism make it possible-for the seal to perform amazing feats.

Chapter Summary

Circulatory Systems: Pumps, Vessels, and Blood

- ▶ The metabolic needs of the cells of small aquatic animals are met by direct exchange of materials with the external medium. The metabolic needs of the cells of larger animals are met by a circulatory system that transports nutrients, respiratory gases, and metabolic wastes throughout the body. Review Figure 49.1
- ▶ In open circulatory systems the blood or tissue fluid leaves vessels and percolates through tissues. Review Figure 49.2
- ▶ In closed circulatory systems the blood is contained in a system of vessels. Review Figure 49.3

Vertebrate Circulatory Systems

- ▶ The circulatory systems of vertebrates consist of a heart and a closed system of vessels containing blood that is separate from the tissue fluid. Arteries and arterioles carry blood from the heart; capillaries are the site of exchange between blood and tissue fluid; venules and veins carry blood back to the heart.
- ▶ The vertebrate heart evolved from two chambers in fishes to three in amphibians and reptiles and four in crocodilians, mammals, and birds. This evolutionary progression has led to an increasing separation of blood flow to the gas exchange organs and blood flow to the rest of the body. Review Pages 868-870
- ▶ In birds and mammals, blood circulates through two circuits: the pulmonary circuit and the systemic circuit.

CIRCULATORY SYSTEMS 885

The Human Heart:Two Pumps in One

- ^ The human heart has four chambers. Valves in the heart prevent the backflow of blood. Review Figure 49.4
- ▶ The cardiac cycle has two phases: systole, in which the ventricles contract; and diastole, in which the ventricles relax. The sequential heart sounds ("lub-dub") are made by the closing of the heart valves. Review Figure 49.5
- ▶ Blood pressure can be measured using a sphygmomanometer and a stethoscope. Review Figure 49.6
- ▶ The autonomic nervous system controls heart rate. Sympathetic activity increases heart rate, and parasympathetic activity decreases it. These actions are due to the effects of norepinephrine and acetylcholine on the rate of depolarization of the membranes of pacemaker cells. Review Figure 49.7
- ▶ The sinoatrial node controls the cardiac cycle by initiating a wave of depolarization in the atria, which is conducted to the ventricles through the atrioventricular node. Review Figure 49.8

^ The EKG records electric potentials resulting from the contraction and relaxation of the cardiac muscles. Review Figure 49.9

The Vascular System: Arteries, Capillaries, and Veins

- ▶ Arteries and arterioles have many elastic fibers that enable them to withstand high pressures. Abundant smooth muscle cells allow these vessels to contract and expand, altering their resistance and thus blood flow. Review Figure 49.10
- ▶ Capillary beds are the site of exchange of materials between blood and tissue fluid.
- ▶ The exchange of fluids between blood and tissues is determined by the balance between blood pressure and osmotic potential in the capillaries. Review Figure 49.12
- ▶ The ability of a specific molecule to cross a capillary wall depends on the architecture of the capillary, the type of substance, and the concentration gradient between the blood and the tissue fluid.
- ▶ A separate system of vessels, the lymphatic system, returns the tissue fluid to the blood.
- ▶ Veins have a high capacity for storing blood. Aided by gravity, by contractions of skeletal muscle, and by the actions of breathing, they carry blood back to the heart. Review Figure 49.13
- ▶ Cardiovascular disease is responsible for about half of all deaths in the United States and Europe. Atherosclerosis and thrombus formation can lead to potentially fatal conditions such as heart attack and stroke. Diet and behavior are the keys to good cardiovascular health. Review Figure 49.14

Blood: A Fluid Tissue

- ▶ Blood can be divided into a plasma portion (water, salts, and proteins) and a cellular portion (red blood cells, white blood cells, and platelets). All of the cellular components are produced from stem cells in the bone marrow. Review Figure 49.15
- ▶ Red blood cells transport respiratory gases. Their production in the bone marrow is stimulated by erythropoietin, which is

produced in response to hypoxia in the tissues. Review Figure 49.16

- Platelets, along with circulating proteins, are involved in clotting responses. Review Figure 49.17
- Plasma is a complex solution that contains gases, ions, nutrient molecules, proteins, and other molecules.

Control and Regulation of Circulation

- Blood flow through capillary beds is controlled by local autoregulation mechanisms, hormones, and the autonomic nervous system. Review Figure 49.18
- Blood pressure is controlled in part by the hormones vasopressin and angiotensin, which stimulate contraction of blood vessels. Review Figure 49.19
- Heart rate is controlled by the autonomic nervous system, which responds to information about blood pressure and blood composition that is integrated by regulatory centers in the brain. Review Figure 49.20
- Diving mammals conserve blood oxygen stores by slowing the heart rate during dives. Review Figure 49.21

For Discussion

1. At the beginning of a race, cardiac output increases immediately before there is any change in blood oxygen or carbon dioxide concentrations. Explain two factors that contribute to this effect. Include the Frank-Starling law in your answer.
2. Explain how the hearts of crocodilians have the advantages of mammalian hearts during exercise but the efficiency of reptilian hearts during rest.
3. A sudden and massive loss of blood results in a decrease in blood pressure. Describe several mechanisms that help return blood pressure to normal.
4. You can describe the cycle of events in a ventricle of the heart by a graph that plots the pressure in the ventricle on the y-axis and the volume of blood in the ventricle on the x-axis. What would such a graph look like? Where would the heart sounds be on this graph? How would the graph differ for the left and the right ventricles?
5. If the major arteries become clogged with plaque and become less elastic because of calcification, the left ventricle must work harder and harder to pump an adequate supply of blood to the body. As a result, the left ventricle can become weakened and begin to fail even though the right ventricle is healthy. A heart attack primarily affecting the left ventricle can have the same effect. This condition is known as congestive heart failure, and commonly leads to fatal pulmonary edema. Explain how left ventricular failure can result in pulmonary edema, and why is it said that this condition creates a vicious circle that makes itself worse rapidly.

50

Animal Nutrition

Recently, The Center for Disease Control has recently determined that one out of every

five people in the United States is obese— at least 30 percent over recommended body mass. This epidemic level of obesity reflects a dramatic increase: Less than 10 years ago, the number was one in eight. The CDC has placed obesity just below smoking as the second largest preventable cause of death. Yet, as a nation, we seem to be more health-conscious than ever before, and at the time of this writing, six diet books are on the New York Times bestseller list. So what is wrong?

Lifestyle changes have played a role. The consumption of "fast food" with a high fat content has risen, snack foods are more prevalent, and people have become more sedentary overall in spite of an increase in planned exercise. Yet different individuals exposed to the same routines and stimuli differ in their propensity to gain weight. Like all other physiological functions, food intake is under genetic and regulatory influences that we are just beginning to understand. In humans, we know that identical twins reared together or apart rarely differ in body mass by more than a few percent. In animals, and now in humans, we know of single-gene mutations that can cause excessive food intake and obesity, and we know of tiny brain areas that when damaged can cause increased or decreased food intake. Can knowledge of nutrition and the physiology of food intake help us fight the epidemic of obesity?

In this chapter we review the nutrients that organisms require for energy, for molecular building blocks, and for specific biochemical functions. We examine briefly the diversity of adaptations for acquiring and ingesting food. Most of the chapter is devoted to how food is digested and absorbed. Then we learn how the body regulates its traffic in metabolic fuels, and return to the quandaries we have just posed about the regulation of food intake and body mass. Last, we raise the issue of environmental toxicology. By taking in nutrients as food, animals also take in com-

A Sizable Problem

Obesity is often caused or compounded by lifestyle. In the United States today, 1 in 5 adults is obese (30 percent or more above recommended weight limits) and liable to have resulting health problems.

pounds that can be toxic. We briefly consider how animals deal with toxic compounds and how human activities that contribute new and highly dangerous toxic compounds to the environment are affecting human health and other organisms in

the environment.

Nutrient Requirements

Animals must eat to stay alive. They eat other organisms— both plants and animals. Since they derive their nutrients from other organisms, they are called heterotrophs. In contrast, autotrophs (most plants, some bacteria, and some protists) trap solar energy through photosynthesis and use that energy to synthesize all of their components. Directly or indirectly, heterotrophs take advantage of—indeed, depend on—the organic synthesis carried out by autotrophs.

Heterotrophs have evolved an enormous diversity of adaptations to exploit, directly or indirectly, the resources made available through the actions of autotrophs (Figure 50.1). In this section we discover how animals use nutrients for energy and to build more complex molecules. We also examine mineral nutrients, such as iron and calcium, and molecules called vitamins that animals need in small quantities.



(a) *Castor canadensis*

(b) *Cyananthus latirostris*



(c) *Eubalaena australis*

Baleen plates

50.1 The Consumers

Heterotrophs have evolved a range of adaptations for exploiting sources of energy, (a) The staple food of the beaver, a mammalian herbivore, is bark. Trees and shrubs felled in the autumn are stored underwater in their lodges (also constructed of felled tree branches), insuring a constant supply of food during the winter, (b) The long bill and hovering flight pattern of the hummingbird, a fluid feeder, enable it to harvest nectar from individual flowers, (c) Filter-feeding right whales consume massive quantities of tiny phytoplankton by filtering ocean water through baleen plates in their mouths, (d) The carnivorous polar bear is a fearsome predator, preying mainly on marine mammals such as seals.

Energy can be measured in calories

In Chapters 6 and 7, we learned that energy in the chemical bonds of food molecules is transferred to the high-energy phosphate bonds of ATP. ATP provides the energy for cellular work. Each conversion of energy from food molecules to ATP and from ATP to cellular work is inefficient; in fact,

most of the energy that was in the food is lost as heat. Even the useful energy conversions eventually are reduced to heat, as molecules that were synthesized are broken down and the energy of movement is dissipated by friction.

(d) *Ursus maritimus*

In time, all the energy that is transferred to ATP from the chemical bonds of food molecules is released to the environment as heat. Therefore, we can talk about the energy requirements of animals and the energy content of food in terms of a measure of heat energy: the calorie. A calorie is the amount of heat necessary to raise the temperature of 1 g of water 1°C. Since this value is a tiny amount of energy in comparison to the energy requirements of many animals, physiologists commonly use the kilocalorie (kcal) as a unit of measure (1 kcal = 1,000 calories). Nutritionists also use the kilocalorie as a standard unit of energy, but they traditionally refer to it as the Calorie, which is always capitalized to distinguish it from the single calorie. (Scientists are gradually abandoning the calorie as an energy unit as they switch to the International System of Units. In this system, the basic unit of energy is the joule: 1 calorie = 4.184 joules.)

The metabolic rate of an animal (see Chapter 40) is a measure of the overall energy needs that must be met by the animal's ingestion and digestion of food. The components of food that provide energy are, fats, carbohydrates, and proteins. Fats yield 9.5 kcal/g, carbohydrates 4.2 kcal/g, and proteins about 4.1 kcal/g. The basal metabolic rate of a human

888 CHAPTER FIFTY

1 glass orange juice 110 kcal

1 piece pizza 133 kcal

3 oz sirloin steak

330 kcal



1 cup shelled peanut 840 kcal

Apple pie à la mode 585 kcal



85 min 22 min 11 min

142 min 37 min 18.5 min

254 min 66 min 33 min

646 min 168 min 84 min

450 min 117 min 58.8 min

50.2 Food Energy and How Fast We Burn It

The energy in kilocalories contained in several common food items is shown at the left. The graph indicates about how long it would take a person with a basal metabolic rate of about 1,800 kcal/day to utilize the equivalent amount of energy while involved in various activities.

(the metabolic rate resulting from all of the essential physiological functions of a resting person) is about 1,300–1,500 kcal/day for an adult female and 1,600–1,800 kcal/day for an adult male. Physical activity adds to this basal energy requirement. For a person doing sedentary work, about 30 percent of total energy consumption is due to skeletal muscle activity, and for a person doing heavy physical labor, 80 percent or more of the total caloric expenditure is due to skeletal muscle activity. Some equivalencies of food, energy, and exercise are shown in Figure 50.2.

Sources of energy can be stored in the body

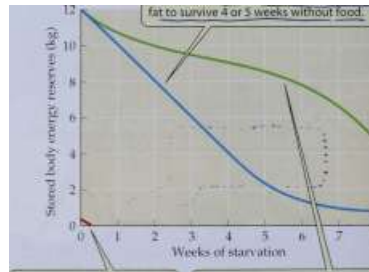
Although the cells of the body use energy continuously, most animals do not eat continuously. Humans generally eat several meals a day, a lion may eat once in several days, a boa constrictor may eat once a month, and hibernating animals may go 5 to 6 months without eating. Therefore, animals must store fuel molecules that can be released as needed between meals.

Carbohydrates are stored in liver and muscle cells as glycogen ("animal starch," see Chapter 3), but the total glycogen store is usually not more than the equivalent of a day's energy requirements. Fat is the most important form of stored

energy in the bodies of animals. Not only does fat have the highest energy content per gram, but it can be stored with little associated water, making it more compact. If migrating birds had to store energy as glycogen rather than fat to fuel their long flights, they would be too heavy to fly! Protein is not used to store energy, although body protein can be metabolized as an energy source of last resort.

If an animal takes in too little food to meet its needs for metabolic energy, it is undernourished, and must make up the shortfall by metabolizing some of the molecules of its own body. Consumption of self is inevitable. The storage compounds glycogen and fat. Protein loss is minimized for as long as possible, but eventually a starving animal uses its own proteins for fuel. The breakdown of body proteins impairs body functions and eventually leads to death. Blood proteins are among the first to go, resulting in loss of fluid to the intercellular spaces (edema; see Chapter 49). Muscles atrophy (waste away), and eventually even air protein is lost. Figure 50.3 shows the course of starvation.

Our major energy reserve is fat; even a person of average body weight has enough fat to survive 4 or 5 weeks without food.



The carbohydrate reserves of our bodies are meager and are depleted by only a single day without food intake.

When most body fat has been exhausted, the only remaining fuel is protein, which is lost at an accelerating rate, with serious consequences, even death.

50.3 The Course of Starvation

In a person subjected to undernutrition, the energy reserves of the body are eventually depleted. Body fat is our major defense against starvation.

Undernourishment is rampant among people in nonindustrialized and war-torn nations, and a billion people—one-sixth of the world's population—are undernourished. (ironically, one cause of life-threatening undernourishment in Western nations is a self-imposed starvation syndrome called anorexia nervosa that results from a psychological aversion to body fat.)

When an animal consistently takes in more food than it needs to meet its energy demands, it is overnourished. The excess nutrients are stored as increased body mass. First, glycogen reserves build up; then additional dietary carbohydrate is stored as fat. Proteins are converted to body fat. In some species, such as hibernators, seasonal overnutrition is an important adaptation for surviving periods when food is unavailable. In humans, however, overnutrition can be a serious health hazard, increasing the risk of high blood pressure, heart attack, diabetes, and other disorders.

Food provides carbon skeletons for biosynthesis

Every animal requires certain basic organic molecules (carbon skeletons) that it cannot synthesize for itself, but needs to build its own complex organic molecules. An example of a required carbon skeleton is the acetyl group (Figure 50.4). Animals cannot make acetyl groups from carbon, oxygen, and hydrogen molecules; they obtain acetyl groups by metabolizing carbohydrates, fats, or proteins.

The acetyl group can be derived from the metabolism of almost any food. It is unlikely ever to be in short supply for an adequately nourished animal. Other carbon skeletons, however, are derived from more limited sources, and an animal can suffer a deficiency of these materials even if its caloric intake is adequate. This state of deficiency is called malnutrition.

Amino acids, the building blocks of proteins, are a good example of carbon skeletons that can be in short supply.

Humans obtain amino acids by breaking down proteins from food and absorbing the resulting amino acids. Another source of amino acids is the breakdown of existing proteins, which are in constant turnover as the tissues of the body undergo normal remodeling and renewal. From these amino acids and ones from food, the body synthesizes its own protein molecules as specified by its DNA.

Animals can synthesize some of their own amino acids by taking carbon skeletons synthesized from acetyl or other groups and transferring to them amino groups ($-NH_2$) derived from other amino acids. But most animals cannot synthesize all the amino acids they need. Each species has certain essential amino acids that must be obtained from food. Different species have different essential amino acids, and in general, herbivores have fewer essential amino acids than carnivores have!

If an animal does not take in one of its essential amino acids, its protein synthesis is impaired. Think of protein synthesis as using a keyboard to write a story. If one letter on the keyboard doesn't function, the story either comes to a stop or has an error in it whenever that letter is needed. In protein synthesis, the story usually comes to a stop, and a

functional protein is not produced.

There are eight essential amino acids that humans must obtain from their food: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. All eight are available in milk, eggs, or meat, but no plant food contains all eight. A strict vegetarian runs a risk of protein malnutrition. An appropriate dietary mixture of plant foods, however, supplies all eight essential amino acids (Figure 50.5). In general, grains are complemented by legumes or by milk or meat. Legumes are complemented by grains, seeds, and nuts. Long before the chemical basis for this complementarity was understood, societies with little access to meat developed healthy dietary practices. Many

Animals use acetyl groups obtained from their food to build more complex organic molecules.

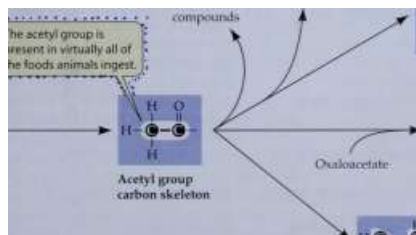
The acetyl group is present in virtually all of the foods animals ingest.

Other compounds

Steroid hormones

Protein,

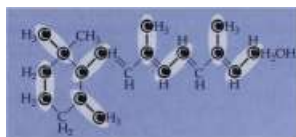
carbohydrate, or fat metabolism



Acetyl group carbon skeleton

50.4 The Acetyl Group Is an Acquired Carbon Skeleton

Animals cannot synthesize the acetyl group for themselves, but they ingest it in their food and use it to synthesize a wide variety of molecules.



Vitamin A

$\text{H}_3\text{C}-\text{COOH}$ "I HO-C-COOH I H($\text{S}-\text{O}$)OH

Citrate

Amino acids, heme, etc.



Palmitic acid (and other fatty acids)

890 CHAPTER FIFTY

-



Grains (corn in tortilla chips)

Eight essential amino acids for humans:

Tryptophan

Methionine

Valine Threonine Phenylalanine Leucine

Isoleucine Lysine



Legumes (beans in bean dip)

«S*

<5>

50.5 A Strategy for Vegetarians

By combining cereal grains and legumes, a vegetarian can obtain all eight essential amino acids.

Central and South American peoples traditionally eat beans

with rice. The combination of beans and rice provides all the essential amino acids.

with grains. The combination of beans and grains provides all the essential amino acids.

Why are dietary proteins completely digested to their constituent amino acids before being used by the body? Wouldn't it be more energy-efficient to reuse some dietary proteins directly? There are several reasons why ingested proteins are not used "as is."

Macromolecules such as proteins are not readily taken up through plasma membranes, but their constituent monomers (such as amino acids) are readily transported.

Protein structure and function are highly species-specific. A protein that functions optimally in one species might not function well in another species. Foreign proteins entering the body directly from the gut would be recognized as invaders and would be attacked by the immune system.

Mineral Elements Required by Animals

ELEMENT

SOURCE IN HUMAN DIET

MAJOR FUNCTIONS

MACRONUTRIENTS

Calcium (Ca)

Chlorine (Cl) Magnesium (Mg) Phosphorus (P) Potassium (K) Sodium (Na) Sulfur (S)

MICRONUTRIENTS

Chromium (Cr)

Cobalt (Co) Copper (Cu)

Dairy foods, eggs, green leafy vegetables, whole

grains, legumes, nuts Table salt (NaCl), meat, eggs, vegetables, dairy

foods Green vegetables, meat, whole grains, nuts,

milk, legumes Dairy foods, eggs, meat, whole grains, legumes,

nuts Meat, whole grains, fruits, vegetables

Table salt, dairy foods, meat, eggs, vegetables

Meat, eggs, dairy foods, nuts, legumes

Meat, dairy foods, whole grains, dried beans,

peanuts, brewer's yeast Meat, tap water

Liver, meat, fish, shellfish, legumes, whole grains, nuts

Found in bones and teeth; blood clotting; nerve and muscle action; enzyme activation

Water balance; digestion (as HCO_3^-); principal negative ion in tissue fluid

Required by many enzymes; found in bones and teeth

Found in nucleic acids, ATP, and phospholipids;

bone formation; buffers; metabolism of sugars Nerve and muscle action; protein synthesis;

principal positive ion in cells Nerve and muscle action; water balance;

principal positive ion in tissue fluid Found in proteins and coenzymes; detoxification of harmful substances

Glucose metabolism

Found in vitamin B₁₂; formation of red blood

cells Found in active site of many redox enzymes and

electron carriers; production of hemoglobin;

bone formation

,

Most animals avoid these problems by digesting food proteins extracellularly and then absorbing the resulting amino acids into the body, where they synthesize new proteins that will function correctly and be recognized as "self" by the immune system.

Using acetyl groups obtained from food, humans can synthesize almost all the lipids required by the body, but we must have a dietary source of essential fatty acids—notably linoleic acid—linoleic acid is an unsaturated fatty acid needed by mammals to synthesize other unsaturated fatty acids such as arachidonic acid, which in turn produces several signaling molecules, including prostaglandins. A deficiency of linoleic acid can lead to problems such as infertility and impaired lactation. Essential fatty acids are also necessary components of membrane phospholipids.

Animals need mineral elements in different amounts

The principal mineral elements required by animals are listed in Table 50.1. Elements required in large amounts are called macrominerals; elements required in only tiny amounts are called micronutrients. Some essential elements are required in such minute amounts that deficiencies are never observed, but these elements are nevertheless essential.

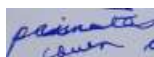
Calcium is an example of a macronutrient. It is the fifth most abundant element in the body; a 70-kg person contains about 1,200 g of calcium. Calcium phosphate is the principal structural material in bones and teeth. Muscle contraction, neuronal function, and many other intracellular functions in animals require calcium. The turnover of calcium in the extracellular fluid is quite high, as bones are constantly being remodeled and calcium is constantly entering and leaving cells. Calcium is lost from the body in urine, sweat, and feces, so it must be replaced regularly from the diet. Humans require about 800 to 1,000 mg of calcium per day in the diet.

Iron is an example of a micronutrient. Iron is found everywhere in the body because it is the oxygen-binding atom in hemoglobin and myoglobin and is a component of enzymes in the electron transport chain. Nevertheless, the total amount of iron in a 70-kg person is only about 4 g, and since iron is recycled so efficiently in the body, we require only about 15 mg per day in our food. In spite of the small amount required, insufficient iron is the most common nutrient deficiency in the world today.

Animals must obtain vitamins from food

Another group of essential nutrients is the vitamins. Like essential amino acids and fatty acids, vitamins are carbon compounds that an animal requires for its normal growth and metabolism, but cannot synthesize for itself. Most vitamins function as coenzymes or parts of coenzymes and are required in very small amounts, compared with the essential amino acids and fatty acids, which have structural roles. The list of vitamins varies from species to species. Most mammals, for example, can make their own ascorbic acid (vitamin C). However, primates (including humans) do not





50.6 A Symptom of Scurvy

Because a vitamin C deficiency weakens connective tissue, the gums bleed, teeth fall out, and blood vessels under the skin break.

have this ability, so for primates ascorbic acid is a vitamin. If we do not get vitamin C in our food, we develop a disease known as scurvy, characterized by bleeding gums, loss of teeth, subcutaneous hemorrhages, and slow wound healing (Figure 50.6). Scurvy was a serious and frequently fatal problem for sailors on long voyages until a Scottish physician, James Lind, discovered that the disease could be prevented if the sailors ate fresh greens or fresh fruit. Eventually the British Admiralty made limes standard provisions for its ships, and ever since British sailors have been called "limeys." The active ingredient in limes was named ascorbic ("without scurvy") acid.

For humans, there are 13 vitamins. They are divided into two groups: water-soluble vitamins and fat-soluble vitamins. Table 50.2 presents these vitamins, their dietary sources, and their functions.

The fat-soluble vitamin D (calciferol), which is essential for the absorption and metabolism of calcium, is a special case because the body can make it. Certain lipids present in the human body can be converted into vitamin D by the action of ultraviolet light on the skin. Thus vitamin D must be obtained in the diet only by individuals with inadequate exposure to the sun, such as people who live in cold climates where clothing usually covers most of the body and where the sun may not shine for long periods of time.

The need for vitamin D may have been an important factor in the evolution of skin color. Human races that are adapted to equatorial and low latitudes have dark skin pigmentation as a protection against the damaging effects of ultraviolet radiation. These peoples generally have extensive skin areas exposed to the sun on a regular basis, so their skin synthesizes adequate amounts of vitamin D. Most human races that became adapted to higher latitudes lost dark skin pigmentation. Presumably, lighter skin facilitates

892 CHAPTER FIFTY



Table 50.2 A Vitamins in the Human Diet

VITAMIN

SOURCE

FUNCTION

DEFICIENCY SYMPTOMS

WATER-SOLUBLE

B₁, thiamin

B₂, riboflavin

Niacin

(nicotinamide, nicotinic acid)

B₆, pyridoxine

Pantothenic acid

Biotin

B₁₂, cobalamin

Folic acid

C, ascorbic acid

FAT-SOLUBLE

A, retinol

D, calciferol

E, tocopherol

K, menadione

Liver, legumes, whole grains,
yeast Dairy foods, organ meats, eggs,
green leafy vegetables Meat, fowl, liver, yeast
Liver, whole grains, dairy foods
Liver, eggs, yeast
Liver, yeast, bacteria in gut Liver, meat, dairy foods, eggs
Vegetables, eggs, liver, whole
grains Citrus fruits, tomatoes, potatoes
Fruits, vegetables, liver, dairy
foods Fortified milk, fish oils,
sunshine Meat, dairy foods, whole grains
Intestinal bacteria, liver
Coenzyme in cellular respiration Beriberi, loss of appetite, fatigue
Coenzyme in cellular respiration
(in FAD and FMN) Coenzyme in cellular
metabolism (in NAD and
NADP) Coenzyme in amino acid
metabolism Found in acetyl CoA
Found in coenzymes Coenzyme in formation of nucleic acids and proteins, and in red blood cell formation
Coenzyme in formation of heme
and nucleotides Aids formation of connective
tissues; prevents oxidation of
cellular constituents
Found in visual pigments
Absorption of calcium and
phosphorus Muscle maintenance, prevents
oxidation of cellular
components Blood clotting
Lesions in corners of mouth, eye irritation, skin disorders
Pellagra, skin disorders, diarrhea, mental disorders
Anemia, slow growth, skin problems, convulsions
Adrenal problems, reproductive problems
Skin problems, loss of hair
Pernicious anemia
Anemia
Scurvy, slow healing, poor bone growth
Night blindness, damage to
mucous membranes Rickets

Anemia

Blood-clotting problems (in newborns)

tates vitamin D production in the relatively small areas of skin exposed to sunlight during the ~shT5ft days of winter. An exception to this correlation between latitude and skin pigmentation is the Iry ujt peoples of the Arcti c. These dark-skinned people obtain plenty of vitamin D from the large amounts of meat and fish oils in t heir diet: for them, ex posur e tO SUNlightj S_P^ nprpscary for nhtaining fhit; v itamin.

When water-soluble vitamins are ingested in excess of bodily needs, they are sirripiy_ diminated in th g_urine. (This is the fate of much of the vitamin C that people take in excessive doses.) The fat-soluble vitamins, however, accumulate in body fat and may build up to toxic levels in the l iver if taken in excess.

Nutrient deficiency diseases

A chronic shortage of any nutrien tpr oduces a characteri stic deficiency disease. If th e deficiency is not remedied, death may follow. An example is kwashiorkor . a disease that results from pr otp'n dpf iriprTry It causes swelling of the extremities, distension of the abdomen (Figure 50.7),breakdown of the immune system, degeneration of the liver, mental retardation, and other problems.

A shortage of any of the vitamins results in specific deficiency symptoms (see Table 50.2). We have already described scurvy, which results from a lack of vitamin C. Another deficiency disease, beriber i : _ wasdirectly involved in the discovery of vitamins. Beriberi means ^xtreme we akness, " It became prevalent in Asia in the nineteenth century, after it became standard practice to mill rice to a high, white polish and discard the hulls that are present in brown rice. A critical observation was that birds—chickens and pigeons—developed beriberi-like symptoms when fed only polished rice. In 1912, Casimir Funk cured pigeons of beriberi by feeding them the discarded hulls.

At the time of Funk's discovery, all diseases were thought to be either caused by microorganisms or inherited. Funk suggested the radical idea that beriberi and some other diseases are dietary in origin and result from deficiencies in specific substances. Funk coined the term "vitamines" because he mistakenly thought that all these substances were amines (compounds with amino groups) vital for life. In 1926, thiamin (vitamin B^—the substance lost in the rice milling process—was theJirst vitamin to be isolated in pure form.

ANIMAL NUTRITION 893



50.7 Kwashiorkor, "The Rejected One"

The swollen abdomen, face, hands, and feet due to edema (fluid retention), as well as the spindly limbs of these children, are hallmarks of serious protein starvation. These symptoms are a result of the body breaking down blood proteins and muscle tissue to obtain needed amino acids.

Deficiency diseases can also result from an inability to absorb or process an essential nutrient even if it is present in the diet. Vitamin B 12 (cobalamin), for example, is present in all foods of animal origin. Since plants neither use nor produce vitamin B 12 , a s trictly vegetari an diet (not supplemented byjrilamuxg\l\s) can lead to a B 19 deficiency d isease ca\\ed(gernicious anerny t, characterized by a failure of r ed blood cells to mature. The most common cause of pernicious anem ia, huwove* , is not a lack of vitamin B 12 in the diet, but an inabi lity to absorb it. Normally, cells in the stomach lining secrete a peptide called intrinsic factor, which binds to vitamin B 12 and makes it possible for it to be absorbed in the ileum of the small intestine. Conditions that damage the stomach lining can therefore cau se an emia.

Inadequate mineral nutrition can also lead to deficiency diseasesvrl^ine^Jbr example, is a constituent of the hor-mone thyronine, w hich is produced in the thyroid gland. If insufficient iodine is obtained in the diet, the thyroid gland grows larger in an attempt to compensate for the inadequate production of thyroxine (see Figure 41.9). The

swelling of the neck that results is called a gcnte r. The introduction of iodiz ed table salt has greatly reduced th e incidence of

goiter in the I Jnitpd States.

Adaptations for Feeding

Heterotrophic organisms can be classified by how they acquire their nutrition. Saprobies (also called saprotrophs or decomposers) are mostly protists and fungi that absorb nutrients from dead organic matter. Detritivores, such as earthworms and crabs, get fed by feeding on dead organic material. Animals that feed on living organisms are predators. They prey on plants, animals, or other animals.

Herbivores and omnivores prey on both. Filter feeders, such as clams and blue whales, prey on small organisms by filtering them from the environment. Fluid feeders include mosquitoes, aphids, and leeches, as well as birds that feed on plant nectar. The anatomical adaptations that enable a species to exploit a particular source of nutrition are usually quite obvious, but physiological and biochemical adaptations can be just as important.

The food of herbivores is often low in energy and hard to digest

Vegetation is frequently coarse and difficult to break down physically, but herbivores must process large amounts of it, since its energy content is low. Most herbivores spend a great part of their time feeding. Many have striking adaptations for feeding, such as the trunk (a flexible, gripping nose) of the elephant or the long neck of the giraffe. Many types of grinding, rasping, cutting, and shredding mouth-parts have evolved in invertebrates for ingesting plant material, and the teeth of herbivorous vertebrates have been shaped by selection to process

coarse plant material. The digestive processes of herbivores can also be quite specialized. The Australian koala, for example, eats nothing but the leaves of eucalyptus trees. Eucalyptus leaves are tough, low in nutrient content, and loaded with pungent, toxic compounds that evolved to protect the trees from predators. Yet the koala's gut can digest and detoxify the leaves and absorb all the nutrients the animal needs from this highly specialized and formidable diet.

Carnivores must detect, capture, and kill prey

The predatory behaviors of many carnivores are legendary. One need only call to mind the hunting skills of hawks, wolves, or any member of the cat family. Carnivores have evolved stealth, speed, large jaws, sharp teeth, and strong gripping appendages. Carnivores also have evolved remarkable means of detecting prey. Bats use echolocation, pit vipers sense infrared radiation from the warm bodies of their prey, and certain fishes detect electric fields created in the water by their prey (see Chapter 45).

Adaptations for killing and ingesting prey are diverse and highly specialized. These adaptations can be especially important when the prey are capable of inflicting damage on the predator. A snake may strike with poisonous fangs,

894 CHAPTER FIFTY



50.8 Adaptations for Feeding

(a) Snakes such as this Texas rat snake (*Elaphe obsoleta*) can ingest large prey (in this case a lizard) by dislocating their jaws, (b) This sea star is eating a clam. While it holds the clam with its arms, enzymes from its everted stomach digest it.

using its venom to immobilize its prey before ingesting it. To swallow large prey, a snake disengages its lower jaw from its joint-with-the-skull (Figure 50.8a). The tentacles of jellyfishes, corals, squid, and octopuses, the long, sticky tongues of frogs and lizards, and the webs of spiders are other fascinating examples of adaptations for capturing and immobilizing prey.

Because some prey items are impossible to ingest, some predators digest their prey externally. Sea stars evert their stomachs (turn them inside out) and digest their molluscan prey while they are still in the invertebrate's shells (Figure 50.8b). Spiders usually prey on insects with indigestible exoskeletons. The spider injects its prey with digestive enzymes and then sucks out the liquefied contents, leaving behind the empty exoskeletons frequently seen in old spider webs.

Vertebrate species have distinctive teeth

Teeth are adapted for the acquisition and initial processing of specific types of foods. Because they are one of the hardest structures of the body, an animal's teeth remain in the environment long after it dies. Paleontologists use teeth to identify animals that lived in the distant past and to deduce what their feeding behavior might have been.

All mammals have a general structure consisting of three layers (Figure 50.9a). An extremely hard material called enamel, composed principally of calcium phosphate, covers the crown of the tooth. The crown and the root contain a layer of bony material called dentine, inside of which is a pulp cavity containing blood vessels, nerves, and the cells that

proHnrrp thp rjpn finp

The shapes and organization of mammalian teeth, however, can be very different, since they are adapted to specific diets (Figure 50.9b). In general, incisors are used for cutting, chopping, or gnawing; canines are used for stab-



bing, ripping, and shredding; and molars and premolars (the cheek teeth) are used for shearing, crushing, and grinding. The highly varied diet of humans is reflected by our multipurpose set of teeth, as is common among omnivores.

Digestion

Most animals digest their food extracellularly. Animals take food into a body cavity that is continuous with the outside environment, into which they secrete digestive enzymes. The enzymes act on the food, reducing it to nutrients that can be absorbed by the cells lining the cavity. Only after they are absorbed by the cells are the nutrients within the body of the animal.

The simplest digestive systems are gastrovascular cavities

that

connect to the outside world through a single opening. An example is the cnidarians, which capture prey using stinging nematocysts and cram it into their gastrovascular cavities with tentacles (see Figures 31.7 and 49.1a). Enzymes in the gastrovascular cavity partly digest the prey. Cells lining the cavity take in small food particles by endocytosis. The vesicles that are created by endocytosis fuse with lysosomes containing digestive enzymes, and intracellular digestion completes the breakdown of the food. Nutrients are released to the cytoplasm as the vesicle breaks down.

Tubular guts have an opening at each end

The guts of most animals are tubular. A mouth takes in food; molecules are digested and absorbed throughout the length of the gut; and solid digestive wastes are excreted through an anus. Different regions in the tubular gut are specialized for particular functions (Figure 50.10). These

functions must be coordinated so that they occur in proper sequence and at appropriate rates to maximize the efficiency of digestion and absorption of nutrients. Keep in mind as we discuss these regions that all locations within the tubular gut are, really, outside the body of the animal. Only by crossing the plasma membranes of cells lining the gut do nutrients enter the body.

(«)

Q All mammalian teeth have a general structure consisting of three layers.

Crown-

Gums'

Root

Cement (holds tooth in bone)



Q An extremely hard material called enamel, composed principally of calcium phosphate, covers the crown of the tooth.

(&) Lower jaw (top view)

Both the crown and the root contain a layer of bony material called dentine...

...within which is a pulp cavity containing blood vessels, nerves, and the cells that produce the dentine.

Nerves and blood vessels

Bone

<S>

50.9 Mammalian Teeth

{ a) A mammalian tooth has three layers: enamel, dentine, and pulp cavity , (b) The teeth of different mammalian species are specialized for different diets.

At the ante rior end of the gut are th e mout h (the opening itself) and Hfec cal ca vity (mouth cavity). Food may be broken up by teeth (in some vertebrates), by the _radula (in snails), or by man dibles (in insects), or somewhat farther along the gut by structures such as the gizzar ds of birds and ea rthworms , where muscular contractions of the gut grind the food together with small stones. Some animals, such as snakes, simply ingest large chunk s of fo od, w itjiittle or no fragmentation.

S tomachs a nd crops are storage chambers that enable animals to ingest relatively large amounts of food and digest it at leisure. In these storage chambers, food may be furt her fragmented and mixed , but diges tion ma y pf-raay not ornir th ere, depe n ding on the specie s. In any case, food

50.10 Compartments for Digestion and Absorption

Most animals have tubular guts that begin with a mouth that takes in food and end in an anus that excretes wastes. Between these two structures are specialized regions for digestion and nutrient absorption; these structures vary from species to species.

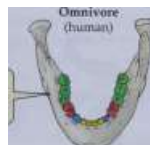
Earthworm

Mouth Crop

Cockroach

Esophagus

Omnivores have a multipurpose set of teeth.



Herbivore

(sheep)

Carnivore

(cat)

Carnivores have greatly enlarged canine teeth for gripping, killing, and tearing their prey.



■ Canines (used for ripping

and tearing) I I Incisors (for cutting)

B Premolars (for shearing) □ Molars (for grinding)



Herbivores use their incisors and canines, which are found far forward on the lower jaw only, to tear leaves off of plants.

"§>

delivered into the next section of gut, the midgut or intestine is well minced and well mixed.

Most materials are digested and absorbed in the midgut. Specialized glands secrete some digestive enzymes into the intestine, and the gut wall itself secretes other digestive enzymes. The hindgut recovers water and ions and stores

undigested wastes, or feces, so that they can be released to the environment at an appropriate time or place. A muscular rectum near the anus assists in the expulsion of feces, the process of defecation.

Within the hindguts of many species are colonies of endosymbiotic bacteria. These bacteria obtain their own nutrition from the food passing through the host's gut while contributing to the digestive processes of the host. Members of the leech genus *Hirudin* for example, produce no enzymes that can digest the proteins in the blood they suck from vertebrates. A colony of gut bacteria produces the enzymes necessary to break down those proteins into amino acids, which are subsequently used by both the leech and

Crop

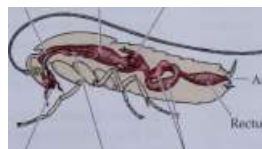
Gizzard

Intestine



Anus

Pharynx Esophagus Gizzard



Rabbit

Salivary glands

Pancreas Cecum Rectum

—Anus

Rectum



Mandibles

Salivary glands

Intestine

Teeth

Esophagus Liver Stomach Large

intestine

896 CHAPTER FIFTY

(i/) Earthworm

(b) Shark

Earthworms have a V longitudinal infolding of the intestinal wall.



Intestine Infolding



Sharks have evolved a spiral valve that increases the surface area of the intestine

(c) Human

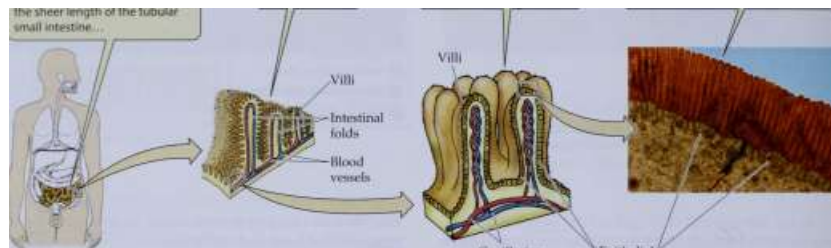


In most vertebrates, an enormous absorptive surface is achieved by the sheer length of the tubular small intestine...

...and the folding of its lining.

Fingerlike villi increase the surface area of these folds...

...and microvilli cover the villi, vastly increasing the absorptive surface area.



Capillaries

50.1 7 Greater Intestinal Surface Area Means More Nutrient Absorption

The guts of most animals have evolved to maximize their surface area.

the bacteria. Some animals rely on microorganisms in their

microbiome to supply them with vitamins

In many animals, the parts of the gut that absorb nutrients have evolved to have enormous surface areas (Figure 50.11a, b). In vertebrates, the wall of the gut is richly folded, with the individual folds bearing legions of tiny fingerlike projections called villi (Figure 50.11c). The cells that line the surfaces of the villi, in turn, have microscopic projections, called microvilli. This provides an enormous surface area for the absorption of nutrients.

Digestive enzymes break down complex food molecules

Protein, carbohydrate, and fat macromolecules are broken down into their simplest units by hydrolytic enzymes. All of these enzymes cleave the chemical bonds of macromolecules through hydrolysis, a reaction that adds a water molecule (see Figure 3.2). Examples of hydrolysis are the breaking of the bonds between adjacent amino acids of a protein or peptide and between adjacent glucose units of a starch.

Digestive enzymes are classified according to the substances they hydrolyze: carbohydrases hydrolyze carbohydrates; proteases, proteins; lipases, lipids; and nucleases, nucleic acids. The process of digestion occurs "outside" (extracellularly)

Epithelial cells

and endo- ("within") indicate where the enzyme cleaves the molecule. An endoprotease hydrolyzes a protein at an internal site along the polypeptide chain, and an exoprotease snips away amino acids at the ends of the molecule.

How can an organism produce enzymes that hydrolyze biological macromolecules without digesting itself? Most digestive enzymes are produced in an inactive form, known as a zymogen, so that they cannot act on the cells that produce them. When secreted into the gut, zymogens are activated by another enzyme or by conditions in the gut (which, as you will remember, is outside the body). The lining of the gut is not digested because it is protected by a covering of mucus.

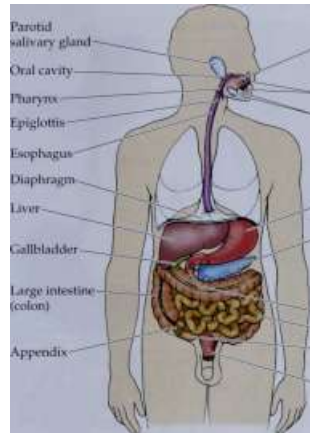
Structure and Function of the Vertebrate Gut

The digestive tract of vertebrates is a tubular gut that runs from mouth to anus (Figure 50.12). Different segments of the gut are specialized for different functions associated with digestion and absorption. In addition, there are several accessory structures that produce and export into the gut compounds that contribute to the digestive process.

Similar tissue layers are found in all regions of the vertebrate gut

The cellular architecture of the vertebrate gut follows a common plan throughout. Four major layers of different

Parotid salivary gland



Tongue

Teeth

Sublingual and submandibular salivary glands

Stomach Pancreas

Large intestine (colon)

Appendix

en 3



Duodenum

Jejunum

Ileum

Rectum

Figure 50.12 The Human Digestive System

Different compartments within the long tubular gut specialize in digesting food, absorbing nutrients, and storing and expelling wastes. Accessory organs contribute digestive juices containing enzymes and other molecules.

Cell types form the wall of the gut (Figure 50.13). These layers differ somewhat from compartment to compartment, but they are always present.

Starting in the cavity, or lumen, of the gut, the first tissue layer is the mucosa. Mucosal cells have secretory and absorptive functions. Some secrete mucus, which lubricates and protects the walls of the gut. Others secrete digestive enzymes, and still others in the stomach secrete hydrochloric acid (HCl). In some regions of the gut, nutrients are absorbed across the plasma membranes of the mucosal cells; the plasma membranes of these absorptive cells have many folds that increase their surface area (see Figure 50.11c).

At the base of the mucosa are some smooth muscle cells, and just outside the mucosa is the second layer of cells, the submucosa. Here we find the blood and lymph vessels that carry absorbed nutrients to the rest of the body. The submucosa also contains a network of nerves; these neurons are

Figure 50.13 Tissue Layers of the Vertebrate Gut

In all compartments of the gut, the organization of the tissue layers is the same, but specialized adaptations of specific tissues characterize different regions.

both sensory (responsible for stomach aches) and regulatory (controlling the various secretory functions of the gut).

External to the submucosa are two layers of smooth muscle cells responsible for the movements of the gut. Innermost is the circular muscle layer with its cells oriented around the gut. Outermost is the longitudinal muscle layer with its cells oriented along the length of the gut. The circular muscles constrict the gut, and the longitudinal muscles shorten the gut. Between the two layers of muscle is another network of nerves that controls the movements of the gut, coordinating the movements of different regions with one another.

Surrounding the gut is a fibrous coat called the serosa. Like other abdominal organs, the gut is also covered and supported by a tissue called the peritoneum.

Peristalsis moves food through the gut

Food entering the mouth of most vertebrates is chewed and mixed with the secretions of salivary glands. A muscular tongue then pushes chewed food toward the back of the buccal cavity. By making contact with the soft tissue at the back of the mouth, the bolus of food initiates a complex series of autonomic reflex actions known as *swallowing*. Stand in front of a mirror and gently touch this tissue at the back of your mouth with a cotton swab. You may gag slightly, but you will also experience an uncontrollable urge to swallow. Swallowing involves many muscles doing a variety of jobs that propel the food through the pharynx (where the mouth cavity and the nasal passages join) and into the esophagus (the food tube). A structure called the epiglottis



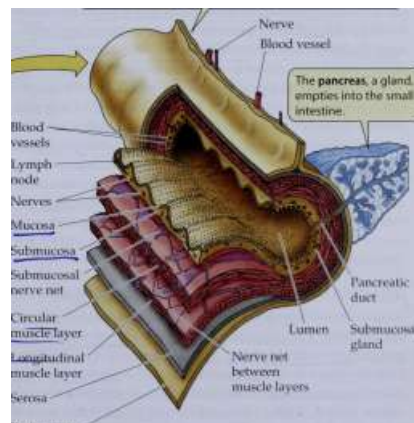
The peritoneum is a membrane that lines the internal wall of the abdominal cavity and covers the abdominal organs.

Nerve Blood vessel

The pancreas, a gland, empties into the small intestine.

Blood vessels

Lymph node



Circular muscle layer

Longitudinal muscle layer

Peritoneum

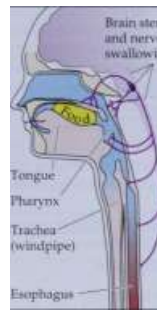
Nerve net between muscle layers

Submucosal gland

898 CHAPTER FIFTY

(a) Swallowing

(b) Peristalsis



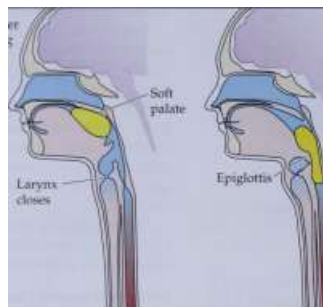
Brain stem reflex center and nerves controlling swallowing

Tongue Pharynx

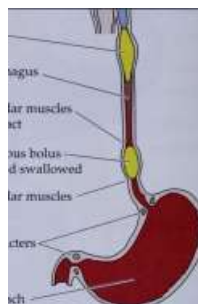
Trachea

(windpipe)

Esophagus



Larynx closes



Food

Esophagus

Circular muscles contract

Previous bolus of food swallowed

Circular muscles relax

Sphincters

Stomach

I Food is chewed and the tongue pushes the bolus of food to the back of the mouth. Sensory nerves initiate the swallowing reflex.

I The soft palate is pulled up as the vocal cords close the larynx.

| The larynx is pulled up and forward and is covered by the epiglottis; the bolus of food enters the esophagus.

50.14 Swallowing and Peristalsis

Food pushed to the back of the mouth triggers the swallowing reflex. Once food enters the esophagus, peristalsis propels it through the gut.

prevents the food from entering the trachea (windpipe) or nasal passages (Figure 50.14).

Once the food is in the esophagus, peristalsis takes over and pushes it toward the stomach. Peristalsis is a wave of smooth muscle contraction that moves progressively down the gut from the pharynx toward the anus. The smooth muscle of the gut contracts in response to being stretched. Swallowing a bolus of food stretches the upper end of the esophagus, and this stretching initiates a wave of contraction that slowly pushes the contents of the gut toward the anus.

The movement of food from the stomach to the esophagus is normally prevented by a thick ring of circular smooth muscle at the junction of the esophagus and the stomach. This ring of muscle, the esophageal sphincter, is normally contracted. Waves of peristalsis cause it to relax enough to let food pass from the esophagus into the stomach. Sphincter muscles are found elsewhere in the digestive tract as well. The pyloric sphincter governs the passage of stomach contents into the intestine. Another important sphincter surrounds the anus.

Digestion begins in the mouth and the stomach

Food is chewed in the mouth, and carbohydrate digestion begins there. The enzyme amylase is secreted with saliva and mixed with the food as it enters the mouth. Amylase hydrolyzes the bonds between the glucose monomers that make up starch. The action of amylase is what makes a piece of bread or cracker taste sweet if you hold it in your mouth long enough.

Peristaltic contractions propel the food to the stomach.

Most vertebrates can rapidly consume a large volume of food, but digesting that food is a long, slow process. The stomach stores the food consumed during a meal. The secretions of the stomach kill microorganisms that are taken in with the food and begin the digestion of proteins.

The major enzyme, produced by the stomach is an endopeptidase called pepsin. Pepsin is secreted as a zymogen called pepsinogen by cells in the gastric glands—deep folds in the stomach lining (Figure 50.15). Other cells in the gastric glands produce hydrochloric acid, and still others near the openings of the gastric glands and throughout the stomach mucosa secrete mucus.

Hydrochloric acid (HCl) maintains the low pH in the stomach.

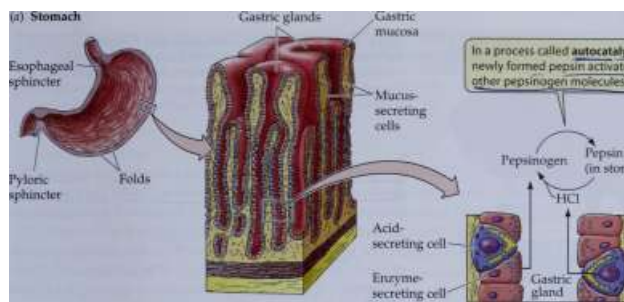
The gastric juice is at a pH between 1 and 3. This low pH activates the conversion of pepsinogen to pepsin, which is achieved by the cleavage of a masking sequence of 44 amino acids from the N-terminal end of the pepsinogen molecule. The conversion is amplified as the newly formed pepsin activates other pepsinogen molecules, a process called autocatalysis. Hydrochloric acid also provides the right pH for the enzymatic action of pepsin. The low pH also helps dissolve the intercellular substances holding the ingested tissues together. Breakdown of the ingested tissues exposes more food surface area to the action of pepsin and eventually other digestive enzymes in the small intestine.

Mucus secreted by the stomach mucosa coats the walls of the stomach and protects them from being eroded and digested by HCl and pepsin. Sometimes, however, the walls of the stomach are exposed to HCl and pepsin; the resulting damage is called an ulcer. It was previously thought that ulcers were mostly due to stress and oversecretion of digestive juices. In recent years, however, it has been discovered that the basis for most ulcers is an infectious bacterium called *Helicobacter pylori*, which has the remarkable

(a) Stomach

Gastric glands

In a process called autocatalysis, newly formed pepsin activates other pepsinogen molecules.



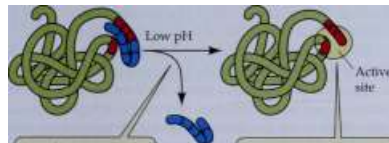
Pyloric sphincter

(in stomach)

{b) Zymogen activation

Inactive zymogen: pepsinogen

Active enzyme: pepsin



A masking sequence is cleaved from the pepsinogen molecule..

Masking sequence

...transforming pepsinogen into the digestive enzyme pepsin.

50.75 The Stomach

(a) The human stomach stores and breaks down ingested food. (b) Cells in the gastric glands secrete hydrochloric acid and the proteolytic enzyme pepsin. Both the glands and gastric mucosa secrete mucus that protects the stomach, (c) Pepsin is secreted as an inactive zymogen, pepsinogen, that is activated by low pH through the cleavage of a masking sequence of amino acids. Active pepsin also activates pepsinogen through autocatalysis.

ability to live in the highly acidic environment of the stomach. Lesions started by the bacterial infection are made worse by HCl and pepsin.

Contractions of the muscles in the walls of the stomach churn its contents, thoroughly mixing them with the stomach secretions. The acidic, fluid mixture of gastric juice and partly digested food in the stomach is called chyme. A few substances can be absorbed from the chyme across the stomach wall, including alcohol (hence its rapid effects), aspirin, and caffeine, but even these substances are absorbed in rather small quantities in the stomach.

Peristaltic contractions of the stomach walls push the chyme toward the bottom of the stomach. These waves of peristalsis cause the pyloric sphincter to relax briefly so that little squirts of the chyme can enter the first region of the intestine. The human stomach empties itself gradually over a period of approximately 4 hours. This slow passage of food enables the intestine to work on a little at a time and extends the digestive and absorptive processes throughout much of the time between meals.

The small intestine is the major site of digestion

In the small intestine the digestion of carbohydrates and proteins continues, and the digestion of fats begins and the absorption of nutrients begins. Although the small intestine takes its name from its diameter, it is a very large organ. The small intestine of an adult human is more than 6 m long; its coils fill much of the lower abdominal cavity (see Figure 50.12). Because of its length, and because of the folds, villi, and microvilli of its lining, its inner surface area is enormous: about 550 m², or roughly the size of a tennis court. Across this surface the small intestine absorbs all the nutrient molecules derived from food. The small intestine

odennurP-is and the ileum carry

has three sections. The initial section

the Sigm Of mrtQt Higp

out 90 percent of the absorption of nutrients

Digestion requires many specialized enzymes, as well as several other secretions. Two accessory organs that are not part of the digestive tract—the liver and the pancreas—pro-

vide many of these enzymes and secretions.

The liver synthesizes a substance called bile from cholesterol. Bile secreted from the liver flows through the hepatic duct to the gallbladder and to the duodenum. Bile reaches the gallbladder through a side branch of the hepatic duct (Figure 50.16). It is stored in the gallbladder until it is needed to assist in fat digestion. When fat enters the duodenum, a hormonal signal causes the walls of the gallbladder to contract rhythmically, squeezing bile back out toward the hepatic duct. Below the branch point to the gallbladder, the hepatic duct is called the common bile duct. Bile from the gallbladder flows down the common bile duct to the duodenum.

To understand the role of bile in fat digestion, think of the oil in salad dressing: it is not soluble in water (it is hydrophobic), and it tends to aggregate together in large globules. The enzymes that digest fat, the lipases, are water-soluble and must do their work in an aqueous medium. Bile stabilizes tiny droplets of fat so that they cannot aggregate into large globules. One end of each bile molecule is soluble in fat (it is lipophilic, or hydrophobic); the other end is soluble in water (it is hydrophilic, or lipophobic). Bile molecules bury their lipophilic ends in fat droplets, leaving their

900 CHAPTER FIFTY



[The liver produces bile,]

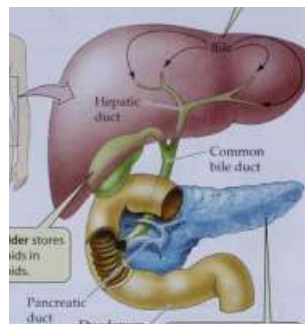
KJ^P



:

\P

The gallbladder stores bile, which aids in digesting lipids.



Pancreatic duct

Duodenum

The pancreas produces digestive enzymes and bicarbonate solution.

(a) Digestion of fats

Q Dietary fats are emulsified into tiny droplets called micelles through the action of bile in the intestinal lumen.

Q Pancreatic lipase works on fats in the micelles to produce free fatty acids and monoglycerides.

(b) Absorption of fats

Intestinal epithelial cell-

El Fatty acids are lipid-soluble and therefore readily dissolve in the plasma membrane and enter the cell.



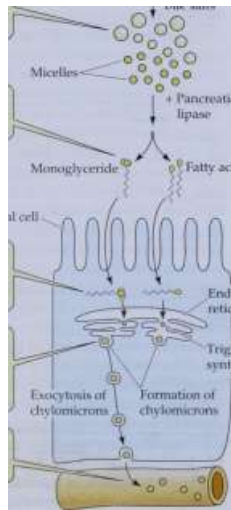
Mixing + bile salts

+ Pancreatic

Fatty acid

Q In the mucosal cell, fatty acids and monoglycerides are resynthesized into triglycerides, and triglycerides are incorporated into chylomicrons.

Q Chylomicrons are passed to the circulatory system through lymphatic vessels.



N

Endoplasmic reticulum

Triglycerides synthesized

50.16 The Ducts of the Gallbladder and Pancreas

Bile produced in the liver leaves the liver via the hepatic duct. Branching off this duct is the gallbladder, which stores bile. Below the gallbladder, the hepatic duct is called the common bile duct and is joined by the pancreatic duct before entering the duodenum.

lipophobic ends sticking out. As a result, they prevent the fat droplets from sticking together. These very small fat particles are called micelles and their small size maximizes the surface area exposed to lipase action (see Figure 50.17). The pancreas is a large gland that lies just beneath the stomach (see Figures 50.12 and 50.16). It functions as both an endocrine (secreting hormones without ducts to the blood and tissue fluid) and an exocrine (secreting other substances through ducts to the outside of the body) gland. Here we will consider its exocrine products, which are delivered to the gut through the pancreatic duct. The pancreatic duct joins the common bile duct just before it enters the duodenum.

The pancreas produces a host of digestive enzymes, including lipases (Table 50.3). As in the stomach, these enzymes are released as zymogens; otherwise they would digest the pancreas and its ducts before they ever reached the duodenum. Once in the duodenum, one of these inactive enzymes, trypsinogen, is activated by enterokinase, which is produced by cells lining the duodenum. This process is similar to the activation of pepsinogen by low pH (see Figure 50.15). Active trypsin can cleave other trypsinogen molecules to release even more active trypsin (another example of autocatalysis). Similarly, trypsin acts on the other zymogens secreted by the pancreas and releases their active enzymes.

The mixture of zymogens produced by the pancreas can be very dangerous if the pancreatic duct is blocked or if the pancreas is injured by an infection or a blow to the abdomen. A few trypsinogen molecules spontaneously converting to trypsin can initiate a chain reaction of enzyme activation that digests the pancreas in a very short period of time, destroying both its endocrine and exocrine functions. The pancreas produces, in addition to digestive enzymes, a secretion rich in bicarbonate ions (HCO_3^-). Bicarbonate ions neutralize the pH of the chyme that enters the duodenum from the stomach. This neutralization is essential because intestinal enzymes function best at a neutral or slightly alkaline pH.

Nutrients are absorbed in the small intestine

Only the smallest products of digestion can be absorbed through the mucosa of the small intestine and passed on to the blood and lymphatic vessels that lie in the submucosa. The final digestion of pro-

Lymphatic vessel

50.17 The Digestion and Absorption of Fats

(a) Dietary fats are broken up by bile into small micelles that present a large surface area to lipase action, (b) The products of fat digestion are absorbed by intestinal mucosal cells, where they are resynthesized into triglycerides and exported to lymphatic vessels.

ANIMAL NUTRITION 901

50.1 Sources and Functions of the Major Digestive Enzymes of Humans

ENZYME

SOURCE

ACTION

SITE OF ACTION

Salivary amylase

Pepsin

Pancreatic amylase

Lipase

Nuclease

Trypsin

Chymotrypsin

Carboxypeptidase

Aminopeptidase

Dipeptidase

Enterokinase

Nuclease

Maltase

Lactase

Sucrase

Salivary glands

Stomach

Pancreas

Pancreas

Pancreas

Pancreas

Pancreas Pancreas Small intestine Small intestine Small intestine Small intestine Small intestine Small intestine Small intestine

Starch → Maltose

Proteins → Peptides; autocatalysis

Starch → Maltose

Fats → Fatty acids and glycerol

Nucleic acids → Nucleotides

Proteins → Peptides; activation of

zymogens Proteins → Peptides Peptides → Peptides and amino acids Peptides → Peptides and amino acids Dipeptides → Amino acids Trypsinogen → Trypsin Nuclease → Nucleotides Maltase → Glucose Lactase → Galactose and glucose Sucrose → Fructose and glucose

Mouth Stomach Small intestine Small intestine Small intestine Small intestine

Small intestine Small intestine Small intestine Small intestine Small intestine Small intestine Small intestine Small intestine

teins and carbohydrates that produces these absorbable products takes place among the micro villi. The (mucosM> fcells jtvith microvilli produc e peptida ses, which cleave larger peptides into tripeptide s, d ipeptide s, and individual amino

arirkjth nt tho mlk ga n.absorb Thp<;p cells also produce the

enzymes maltase, la ctase, a nd sucrase, which cleave the common disaccharides into their constituent, absorbable monosaccharides —gluc ose, gala ctose, and fru ctose .

Many humans stop producing the enzyme lactase around the age of 4 years and thereafter have difficulty digesting lactose, which is the sugar in milk. Lactose is a disaccharide and cannot be absorbed without being cleaved into its constituent units, glucose and galactose. If a substantial amount of lactose remains unabsorbed and passes into the large intestine, its metabolism, by bacteria in the large intestine causes abdominal cramps, gas, and diarrhea.

The mechanisms by which the cells lining the intestine absorb nutrient molecules and inorganic ions are diverse and not completely understood. Many inorganic ions are actively transported into the cells. Carrier proteins exist for sodium and iron. Carriers also exist for certain classes of amino acids and for glucose and galactose, but their activity is much reduced if active sodium transport is blocked. Sodium ions move from the gut contents into the mucosal cells and are then actively transported from the mucosal cells into the submucosa. To diffuse into a mucosal cell, a sodium ion binds to a symport in the mucosal cell membrane. The symport also binds a nutrient molecule such as glucose or an amino acid. The diffusion of the sodium ion, driven by a concentration gradient, therefore drives the absorption of the nutrient molecule. This mechanism is called sodium cotransport.

The absorption of the products of fat digestion does not involve carrier proteins (Figure 50.17). Lipases break down fats into fatty acids and monoglycerides, which are lipid-soluble and are thus able to pass through the plasma membranes of the microvilli and diffuse into the mucosal cells. Once in the cells, the fatty acids and monoglycerides are resynthesized into triglycerides, combined with cholesterol and phospholipids and coated with protein to form water-soluble chylomicrons, which are really little parcels of fat. Rather than entering the blood directly, the chylomicrons pass into the lymphatic vessels in the submucosa. They then flow through the lymphatic system and enter the bloodstream through the thoracic duct. After a meal rich in fats, the chylomicrons can be so abundant in the blood that they give it a milky appearance.

The bile that emulsifies the fats is not absorbed along with the monoglycerides and the fatty acids, but shuttles back and forth between the gut contents and the microvilli. In the ileum, bile is actively reabsorbed and returns to the liver via the bloodstream. As noted earlier, bile is synthesized in the liver from cholesterol. Cholesterol comes from food, but it is also synthesized by liver cells and gut cells.

As we learned in Chapter 4, high cholesterol levels contribute to arterial plaque formation and therefore to cardiovascular disease. The body has no way of breaking down excess cholesterol, so high dietary intake or high levels of synthesis create problems. One major way that cholesterol leaves the body is through the elimination of unabsorbed bile in the feces. The rationale for including certain kinds of fiber in the diet is that the fiber binds bile, decreases its reabsorption in the ileum, and thus helps to lower blood cholesterol levels.

Water and ions are absorbed in the large intestine

Peristalsis gradually pushes the contents of the small intestine into the large intestine, or colon. The rate of peristalsis

902 CHAPTER FIFTY

- « ^



is controlled so that food passes through the small intestine slowly enough for digestion and absorption to be complete, but quickly enough to ensure an adequate supply of nutrients for the body. Most of the available nutrients have been removed from the material that enters the colon, but the material contains a lot of water and inorganic ions.

The colon absorbs water and ions, producing semisolid feces from the slurry of indigestible materials it receives from the small intestine. Absorption of too much water in the colon can cause constipation. The opposite, diarrhea, results if too little water is absorbed or if water is secreted into the colon. (Both constipation and diarrhea can be induced by toxins from certain microorganisms.) Feces are stored in the last segment of the colon and are periodically excreted.

Immense populations of bacteria live within the colon. One of the resident species is *Escherichia coli*, the bacterium that is so popular among researchers in biochemistry, genetics, and molecular biology. This inhabitant of the colon lives on matter indigestible to humans and produces some products useful to its host. Vitamin K and biotin, for example, are synthesized by *E. coli* and absorbed through the wall of the colon. Excessive or prolonged intake of antibiotics can lead to vitamin deficiency because the antibiotics kill the normal intestinal bacteria at the same time they are killing the disease-causing organisms for which they are intended. The intestinal bacteria produce gases such as methane and hydrogen sulfide as by-products of their largely anaerobic metabolism. Humans expel gas after eating beans because the beans contain certain carbohydrates that bacteria—but not humans—can break down.

The large intestine of humans has a small, fingerlike pouch called the appendix, which is best known for the trouble it causes when it becomes infected. The human appendix plays no essential role in digestion, but it does contribute to immune system function. It can be surgically removed without serious consequences. The part of the gut that , »LV-- »



Herbivores have special adaptations to digest cellulose

Cellulose is the principal organic compound in the diets of herbivores. Most herbivores, however, cannot produce cellulolytic enzymes that hydrolyze cellulose. Exceptions include silverfish (well known for eating books and stored papers), earthworms, and shipworms. Other herbivores, from termites to cattle, rely on microorganisms living in their digestive

tracts to digest cellulose for them.

The digestive tracts of ruminants (cud chewers) such as cattle, goats, and sheep are specialized to maximize the benefits of their endosymbiotic microorganisms. In place of the usual mammalian stomach, ruminants have a large, four-chambered organ (Figure 50.18). The first two chambers, the rumen and the reticulum, are packed with anaerobic microorganisms that break down cellulose. The ruminant periodically regurgitates the contents of the rumen (the cud) into the reticulum for chewing. When the more thoroughly ground-up vegetable fibers are swallowed again, they present more surface area to the microorganisms for their digestive actions.

The microorganisms in the rumen and reticulum metabolize cellulose and other nutrients to simple fatty acids, which become nutrients for the host. In addition, the microorganisms themselves provide an important source of protein for the host. The plant materials ingested by a ruminant are a poor source of protein but they contain inorganic nitrogen that the microorganisms use to synthesize their own amino acids. A cow can derive more than 100 g of protein per day from digestion of its endosymbiotic microorganisms.

Carbon dioxide and methane are by-products of the fermentation of cellulose carried out by microorganisms. A single cow can produce and belch 400 liters of methane a day. Methane is the second most abundant of the "greenhouse gases" whose concentration in the atmosphere is increasing,

forms the appendix in humans. The food leaving the rumen

forms the much larger cecum in "carries with it enormous

, , , , r . rr . 10 , numbers of the cellulose-

herbivores (see Figure 50.10), (, ■ ■

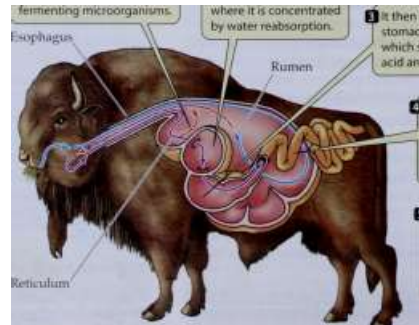
-***^ v o " fermenting microorganisms.

where it functions, as we will see, in cellulose digestion. As our primate ancestors evolved to exploit diets less rich in indigestible cellulose, the cecum no longer served an essential function and gradually became vestigial (reduced to a trace).

| This mixture passes through the omasum, where it is concentrated by water reabsorption.

50.18 The Ruminant Stomach

Four specialized stomach compartments enable ruminants to digest and subsist on protein-poor plant material.



It then enters the true stomach, the abomasum, which secretes hydrochloric acid and proteases.



Reticulum

The microorganisms are killed by the acid, digested by the proteases, and passed on to the small intestine for further digestion and absorption.

The rate of multiplication of microorganisms in the rumen is great enough to offset their loss, so a well-balanced, mutually beneficial relationship is maintained.

ANIMAL NUTRITION 903

and domesticated ruminants are second only to industry as a source of methane gas.

The food leaving the rumen carries with it enormous numbers of the cellulose-fermenting microorganisms. This mixture passes through the omasum, where it is concentrated by water absorption. It then enters the true stomach, the abomasum, which secretes hydrochloric acid and proteases. The microorganisms are killed by the acid, digested by the proteases, and passed on to the small intestine for further digestion and absorption. The rate of multiplication of microorganisms in the rumen is great enough to offset their loss, so a well-balanced, mutually beneficial relationship is maintained.

Some mammalian herbivores other than ruminants have microbial farms and cellulose fermentation. In a branch off the large intestine called the cecum. Rabbits and hares are good examples. Since the cecum empties into the large intestine, the

absorption of the nutrients produced by the microorganisms is inefficient and incomplete. Therefore, some of these animals re-ingest some of their own feces, a behavior known as coprophagy. Coprophagous species usually produce two kinds of feces, one consisting of pure waste (which they discard), and one consisting mostly of cecal material, which they re-ingest directly from the anus. As this cecal material passes through the stomach and small intestine, the nutrients it contains are digested and absorbed.

Control and Regulation of Digestion

The vertebrate gut is an assembly line in reverse—a disassembly line. As with a standard assembly line, control and coordination of sequential processes is critical. Both neural and hormonal controls govern gut functions.

Autonomic reflexes coordinate functions in different regions of the gut

Everyone has experienced salivation stimulated by the sight or smell of food. That response is an autonomic reflex, as is the act of swallowing following tactile stimulation at the back of the mouth. Many autonomic reflexes coordinate activities in different regions of the digestive tract. Loading the stomach with food, for example, stimulates increased activity in the colon, which can lead to a bowel movement.

The digestive tract is unusual in that it has an intrinsic

nerve

called the

enteric nervous system. In addition to autonomic

reflexes involving the CNS, such as salivation and swallowing, neural messages can travel from one region of the digestive tract to another without being processed by the CNS.

Hormones control many digestive functions

Several hormones control the activities of the digestive tract and its accessory organs (Figure 50.19). The first hormone ever discovered came from the duodenum; it was called secretin because it caused the pancreas to secrete digestive juices. We now know that secretin is one of several hormones that control pancreatic secretion; specifically, secretin stimulates the pancreas to secrete a solution rich in bicarbonate ions.

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Secretin

Release of bile from gallbladder

Emulsifies fats

\

Release of

digestive

enzymes from

pancreas

Digestion of food

Release of

bicarbonate

solution

from pancreas

7

Neutralizes acid

50.19 Hormones Control Digestion

Several hormones are involved in feedback loops that control the sequential processing of food in the digestive tract.

In response to the presence of fats and proteins in the chyme, the mucosa of the small intestine secretes c cH oJecys- tokenin, a hormone that stimulates the gallbladder to release bile and the pancreas to release digestive enzymes. Cholecystokinin and secretin also slow the movements of the stomach, which slows the delivery of chyme into the small intestine.

The stomach secretes a hormone..called \wedge gastrin into the blood. Cells in the lower region of the stomach release gastrin when they are stimulated by the presence of food. Gastrin circulates in the blood until it reaches cells in the upper areas of the stomach wall, where it stimulates the secretions and movements of the stomach. Gastrin release is inhibited when the stomach contents become too acidic—another example of negative feedback.

Control and Regulation of Fuel Metabolism

Most animals do not eat continuously. When they do eat, food is present in the gut and nutrients are being absorbed for some period of time after the meal, called the absorp-

904 CHAPTER FIFTY

tive period. Once the stomach and small intestine are empty \wedge nutrients are no longer \wedge in \wedge g \wedge rh \wedge H During this postabsorptive period, the continuous processes of energy metabolism μ \ biosynthesis must run on internal reserves. Nutrient traffic must be controlled so that reserves accumulate during the absorptive period are used appropriately during the postabsorptive period.

The liver directs the traffic of fuel molecules

The liver directs the traffic of the nutrients that fuel metabolism. When nutrients are abundant in the blood, the liver stores them in the forms of glycogen and fats. The liver also synthesizes blood plasma proteins from circulating amino acids. When the availability of fuel molecules in the blood declines, the liver delivers glucose and fats back to the blood. The \wedge Tver has a n enormous capacity to interconvert fuel molecules. Liver cells can convert monosaccharides into ei-

ther glycogen or fat, and vice versa. The liver can also convert certain amino acids and some other molecules, such as pyruvate and lactate, into glucose—a process called gluco-neogenesis. The liver is also the major controller of fat me-'tabollsm through its production of lipoproteins.

Lipoproteins:The good, the bad, and the ugly

In the intestine jfile solves the problem of processing hydrophobic fats in an aqueous medium. The transportation of fats in the circulatory system presents the same problem, and $\text{d l i poBmtej n sjar e}$ the solution . A lipoprotein is)a parti cle made u p of a cor eof fat and choleste rol and a cove ring of protein th at makes it water-solu ble. The largest lipoprotein particles are the chylomicrons produced by the mucosal cells of the intestine, which transport dietary fat and cholesterol into the circulation (see Figure 50.17). As the chylomicrons circulate through the liver and to adipose (fat) tissues throughout the body, receptors on the capillary walls recog-

Increase in circulatory insulin

Stimulates pancreas to secrete insulin

Uptake of glucose by cells

Increase in blood glucose

*



Decreases blood glucose

Decrease in blood glucose

4



nize their protein coats, and lipases begin to hydrolyze the fats, which are then absorbed into liver or fat cells. Thus the protein coat of the lipoprotein both makes it water-soluble and serves as an "address" that directs it to a specific tissue. Lipoproteins other than chylomicrons originate in the liver and are classified according to their density. Fat has a low density (it floats in water), so the more fat a lipoprotein contains, the lower its density.

► Very-low-density lipoproteins (VLDL) produced by the liver contain mostly triglyceride fats that are being transported to fat cells in tissues around the body.

► Low-density lipoproteins (LDL) consist of about 50 to 60 percent cholesterol, which they transport to tissues' around the body for use in biosynthesis and for storage.

► High-density lipoproteins (HDL) serve as acceptors of cholesterol (they consist of about 25 percent cholesterol) and are believed to remove cholesterol from tissues and carry it to the liver, where it can be used to synthesize bile.

Because of their differing functions in cholesterol regulation, LDL is sometimes called "bad cholesterol" and HDL "good cholesterol"—designations that are somewhat controversial. However, we do know that a high ratio of LDL to HDL in a person's blood is a risk factor for atherosclerotic heart disease. Cigarette smoking lowers HDL levels, and regular exercise increases them.

Fuel metabolism is controlled by hormones

During the absorptive period, blood glucose levels are high as carbohydrates are digested and absorbed. During this time, the liver takes up glucose from the blood and converts it to glycogen and fat, fat cells take up glucose from the blood and convert it to stored fat, and the cells of the body preferentially use glucose as their metabolic fuel.

During the postabsorptive period these processes are reversed. The liver breaks down glycogen to supply glucose to the blood, the liver and the adipose tissues supply fatty acids to the blood, and most of the cells of the body preferentially use fatty acids as their metabolic fuel.

One tissue that does not switch fuel sources during the postabsorptive period is the nervous system. The cells of the nervous system require a constant supply of glucose. Even though the nervous system can use other fuels to a limited extent, its overall dependence on glucose is the reason it is so important for other cells of the body to shift to fat metabolism during the postabsorptive period. This shift preserves the available glucose and glycogen stores for the nervous system, for as long as possible.

What directs the traffic in fuel molecules? Insulin and glucagon, two hormones produced and released by the pancreas, are responsible for controlling the metabolic directions that fuel molecules take (Figure 50.20). The most

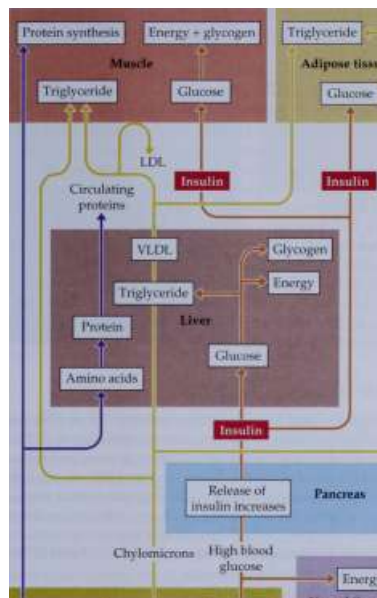
50.20 Regulating Glucose Levels in the Blood

Insulin and glucagon maintain the homeostasis of circulatory glucose.

Metabolic energy Fat synthesis Glycogen synthesis

\

(a) Fuel traffic during the absorptive period



Release of insulin increases

Chylomicrons High blood glucose

Energy

I

Amino acids

Triglycerides and fatty acids

Glucose

Neural tissue

Gut

important of these hormones is ^{insulin} which is produced in response to high blood glucose

The pancreas releases insulin into the circulatory system when blood glucose rises above the normal postabsorptive level. Insulin facilitates the entry of glucose into most cells of the body. When insulin is present, most cells burn glucose as their metabolic fuel, fat cells use glucose to make fat, and liver cells convert glucose to glycogen and fat.

As soon as blood glucose falls back to postabsorptive levels, insulin release diminishes rapidly, and the entry of glucose into cells other than those of the nervous system is inhibited. Without a supply of glucose, cells switch to using glycogen and fat as their metabolic fuels. In the absence of insulin, the liver and fat cells stop synthesizing glycogen and fat and begin breaking them down. As a result, the liver supplies glucose to the blood rather than taking it from the blood, and both the liver and the adipose tissues supply fatty acids to the blood.

(b) Fuel traffic during the postabsorptive period

Protein

Glycogen

Energy

Muscle

Amino acids

Fat

LDL

~

V

Glucagon

t

No insulin

I

Free

fatty

acids

No insulin

Glycogen

VLDL

Fatty acids

Glucose

Triglyceride

Liver

Gluconeogenesis

► Amino acid:



> Blood glucose

Insulin release diminishes

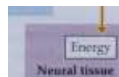
(glucagon is released

secondarily)

Pancreas

Low blood glucose

= Triglycerides and fatty acids -► = Glucose and glycogen -► = Proteins and amino acids



50.21 Fuel Molecule Traffic during the Absorptive and Postabsorptive Periods

Insulin promotes glucose uptake by liver, muscle, and fat cells during the absorptive period. During the postabsorptive period, the lack of insulin blocks glucose uptake by these same tissues and promotes fat and glycogen breakdown to supply metabolic fuel.

glucose

The pancreas releases glucagon when the blood glucose concentration falls below the normal postabsorptive level. Glucagon has the opposite effect of insulin: it stimulates liver cells to break down glycogen and to carry out gluconeogenesis. Thus, under the influence of glucagon, the liver produces glucose and releases it into the blood.

The traffic of fuel molecules during the absorptive and postabsorptive periods is summarized in Figure 50.21, which indicates the steps controlled by insulin and glucagon. During the absorptive period, all fuel molecules move toward storage, and glucose is the preferred energy source for all cells. During the postabsorptive period, most cells switch to metabolizing their own glycogen reserves, while the blood glucose reserves are saved for the nervous system. The level of circulating glucose is maintained through glycogen breakdown and gluconeogenesis.

906 CHAPTER FIFTY

The Regulation of Food Intake

At the beginning of this chapter we noted that obesity is a major health issue in the United States. People spend billions of dollars every year on schemes to lose weight, but the problem increases. A simple rule—take in fewer calories than your body burns, but maintain a balanced diet—should solve the problem, but it doesn't. Why? As we noted, social and lifestyle factors play a major role, but these factors play out against a genetic and regulatory background.

The amount of food an animal eats is governed by its sensations of hunger and satiety, and these sensations are influenced by a region of the brain called the hypothalamus. If a region in the middle of the hypothalamus of rats, called the ventromedial hypothalamus, is damaged, the animals will increase their food intake and become obese. If a different region of the hypothalamus, called the lateral hypothalamus, is damaged, rats will decrease their food intake and become skinny. In both cases the rats eventually reach a new equilibrium body weight, which they maintain. Thus, regulation is maintained, but the level of regulation has been changed. Other brain regions have also been implicated in control of hunger and satiety.

In Chapter 40 we learned that regulation involves feedback information and a means of comparing that information with a set point. There is some evidence that cells in the hypothalamus and in the liver are sensitive to the levels of blood glucose and insulin with high levels stimulating satiety and low levels stimulating hunger. There is even stronger evidence, however, that signals from fat metabolism influence hunger and satiety.

A single-gene mutation in mice, when present in the homozygous condition, results in mice that eat enormous amounts of food and become obese (Figure 50.22). Using genetics terms, these mice are called *ob/ob* mice due to their double dose of the recessive "obese" gene. The wild-type *ob*



50.22 A Single-Gene Mutation Leads to Obesity in Mice

Leptin serves as a negative feedback signal to the brain to limit food intake. The fat cells of the *ob/ob* mouse (left) do not produce leptin. The wild-type mouse (right) does produce leptin and does not become obese when kept under the same conditions as the *ob/ob* mouse.

gene codes for a protein that has been named leptin (from the Greek leptos, "thin"). When leptin was injected into *ob/ob* mice, they ate less and lost body fat. Leptin is produced by fat cells and circulates in the blood. Receptors for leptin are found in the regions of the hypothalamus that are involved in control of hunger and satiety. It seems that leptin signals the brain about the status of the body fat reserves.

Could leptin be used to reduce human obesity? In a very few cases, obese humans do not produce the hormone leptin, and injections of leptin can curb their appetites and enable them to lose body mass. Most obese humans, however, have higher than normal circulating levels of leptin. It is likely that they have receptors with reduced sensitivity. Leptin appears to be one important feedback signal in the regulation of food intake. Understanding the actions of leptin in normal and obese individuals might provide a partial answer as to why some individuals find it easier than others to avoid excessive food intake and increases in body fat. Additional feedback signals are most certainly involved.

Toxic Compounds in Food

Plant and animal tissues contain nutrients, but as we have seen, they can also contain toxic compounds (Figure 50.23). Some mushrooms, for example, contain poisons and hallucinogens; some mollusks, fishes, and amphibians contain neurotoxins; some plants contain compounds that stimulate or depress the heart; and of course, tobacco contains nicotine, poppies contain opium, and marijuana contains tetrahydrocannabinol. Ingesting many plant and animal tissues, therefore, can be dangerous.

Human activities add millions of tons of synthetic toxic compounds to our environment every year, making the problem worse. Many of these compounds enter the air we breathe and the water we drink, as well as the food we eat. A whole new field, called environmental toxicology, has developed to address the problems of poisons in the environment.

Some toxins are retained and concentrated in organisms

The physical and chemical properties of a toxic compound affect its retention within a biological system. If a compound can dissolve in water, it may be quickly metabolized (and thus detoxified) because it is easily accessible to the wide variety of enzymes that can break down complex molecules in food.

In addition to being broken down or metabolized, many water-soluble compounds can be filtered out of the blood by the kidneys, and therefore do not accumulate in the body. That is why urine tests are used to detect illegal drug use by athletes and other individuals. However, some potentially dangerous water-soluble compounds can be incorporated into the body and disrupt normal functions. An example is lead, which can replace iron in blood and calcium in bone.

Lipid-soluble compounds are usually metabolized more slowly than water-soluble compounds, and they are often



Papaver somniferum

Takifugu rubripes

50.23 Toxins Occur Naturally

Many animals and plants contain dangerous, potentially deadly toxins, (a) Opium poppies are the source of morphine and heroin. (b) The puffer fish is a delicacy in Japanese sushi restaurants; restaurants that serve it have special licenses affirming their chef's ability to prepare the fish so that its neurotoxins do not endanger diners.

stored in the body for a long time because they dissolve in the lipids of membranes and adipose tissues. Lipid-soluble compounds can accumulate in the body and reach very high concentrations. Even compounds that are beneficial or neutral to the body at low concentrations, such as fat-soluble vitamins, can become toxic at high concentrations.

Some toxins can bioaccumulate in the environment

Some lipid-soluble toxins, including many pesticides, can bioaccumulate in the environment; that is, they can become more and more concentrated in predators that eat contaminated prey. The pesticide load is passed up the food chain from prey to predator, growing increasingly concentrated in the tissues of each consumer in turn. In the top predator, the pesticide may be concentrated thousands or millions of times. Many top predators show high levels of pesticides and other synthetic chemicals in their tissues. Long-lived species, such as eagles or bears, are particularly at risk for heavy body burdens of accumulated pesticides because they have many years to accumulate them. Bioaccumulated toxins may be responsible for high rates of cancer and infertility in some wildlife populations.

A well-documented case was the effect of the pesticide DDT on predatory bird populations. Bioaccumulated DDT caused extreme thinning of eggshells and therefore high mortality of embryos (Figure 50.24). As a result, many species of predatory birds, such as ospreys, eagles, and falcons, became endangered. Since the banning of DDT use in the United States, these species have been recovering. Scientists and policy-makers are now setting standards for and researching alternatives to such pesticides to decrease the amounts of synthetic toxins bioaccumulating within natural systems. Since humans are at the top of the food chain, eating at all levels, we must consider the risks of our own exposure to these toxins.

ANIMAL NUTRITION 907

The body cannot metabolize many synthetic toxins

How does the way that the body handles synthetic chemicals differ from the way it handles natural chemicals? In many cases, the detoxification systems that metabolize natural chemicals can also metabolize synthetic chemicals, breaking them apart and eliminating them through the urine. Enzymes called cytochrome P450s are responsible for much of this detoxification.

P450s are less specific in their abilities to bind substrates than are most enzymes. Thus, each P450 can catalyze reactions with a wide range of compounds, and there are many P450s. Phase I P450s make small chemical changes to the substrate, such as adding a —OH group or a —SO₃ group, which prepares the substrate for a second reaction. Phase II P450s use the —OH or —SO₃ group to attach a hydrophilic group onto the substrate, which facilitates the elimination of that substrate from the body. Few natural compounds can escape the P450s, even when the body encounters them for the first time.

Some synthetic chemicals, however, fall outside the range of structures that P450s and other enzymes can metabolize. When such chemicals are lipophilic, they bioaccumulate, and any biological effect they have is greatly magnified. If a synthetic

chemical that cannot be metabolized is structurally similar to a hormone, that synthetic chemical may activate the hormonal signaling pathway within target cells. Whereas the natural hormonal signal can be turned off, the synthetic hormone cannot be, and control of function is lost.



50.24 DDT Affects Bird Eggs

Before its use was banned, the bioaccumulation of DDT in birds resulted in severe thinning of the eggshells of many species, with resulting population declines. This brown pelican egg cracked open long before the embryo inside was ready to hatch.

908 CHAPTER FIFTY

One example of a class of synthetic chemicals that appear to mimic hormones in animals is the polychlorinated biphenyls, PCBs. PCBs were produced extensively for use as an insulating fluid in electrical transformers from the 1930s until recently. PCBs are chemically stable, lipophilic, and are now found throughout the environment. They have been shown to bioaccumulate, reaching dangerously high levels in fish from contaminated waters such as the Great Lakes.

The biological response to exposure to PCBs in the diet varies among species. In rhesus monkeys, PCBs altered reproductive cycles, reduced weight gain in infants, depressed immune system responsiveness, and increased the incidence of death in developing embryos. In communities around the Great Lakes, studies have indicated cognitive impairment in children of mothers with a high body burden of PCBs, probably from eating fish caught in the Great Lakes. Studies of animals and of humans that have been exposed accidentally to high levels of PCBs have shown that effects are slow to reverse, lasting from several months to a year.

The risks of PCBs and DDT are now clear, but it is usually difficult to make a causal connection between a toxin in the environment and specific health effects in a population. Environmental toxicologists must be able to study large populations, use powerful statistics, and do controlled laboratory studies to obtain evidence that will support policy changes to stop and reverse the effects of synthetic environmental toxins.

Chapter Summary

Nutrient Requirements

- ▶ Animals are heterotrophs that derive their energy and structural building blocks from food, and therefore ultimately from autotrophs.
- ▶ Carbohydrates, fats, and proteins in food supply animals with metabolic energy. A measure of the energy content of food is the calorie. Excess caloric intake is stored as glycogen and fat. Review Figure 50.2
- ▶ An animal with insufficient caloric intake is undernourished and must metabolize its stored glycogen, fat, and finally its own protein for energy. Overnutrition in humans can be a serious health hazard. Review Figure 50.3
- ▶ For many animals, food provides essential carbon skeletons that they cannot synthesize themselves. Review Figure 50.4
- ▶ Humans require eight essential amino acids in their diet. All are available in milk, eggs, or meat, but not in all vegetables. Thus, vegetarians must eat a mix of foods. Review Figure 50.5
- ▶ Different animals need mineral elements in different amounts. Macronutrients, such as calcium, phosphorus, sodium chloride, and iron, are needed in large amounts. Micronutrients, such as iron, copper, magnesium, and zinc, are needed in small amounts. Review Table 50.1
- ▶ Vitamins are organic molecules that must be obtained in food. Review Table 50.2
- ▶ Malnutrition results when any essential nutrient is lacking from the diet. Lack of any essential nutrient causes a deficiency disease. Review Table 50.2

Adaptations for Feeding

- ▶ Animals can be characterized by how they acquire nutrition: Saprotrophs and detritivores depend on dead organic matter, filter feeders strain the environment for small food items, herbivores eat plants, and carnivores eat animals.
- ▶ Behavioral and anatomical adaptations reflect feeding types. In vertebrates, teeth have evolved to match the diet. Review Figure 50.9

Digestion

- Digestion involves the breakdown of complex food molecules into monomers that can be absorbed and utilized by cells. In most animals, digestion is extracellular and external to the body, taking place in a tubular gut that has different regions specialized for different digestive functions. Review Figure 50.10
- Absorptive areas of the gut are characterized by a large surface area. Review Figure 50.11
- Hydrolytic enzymes break down proteins, carbohydrates, and fats into their monomeric units. To prevent the organism itself from being digested, these enzymes are released as inactive zymogens, which become activated when secreted into the gut.

Structure and Function of the Vertebrate Gut

- The cells and tissues of the vertebrate gut are organized in the same way throughout its length. The innermost tissue layer, the mucosa, is the secretory and digestive surface. The submucosa contains secretory cells and glands, blood and lymph vessels, and nerves. External to the submucosa are two smooth muscle layers (circular and longitudinal) that move food through the gut. Between the two muscle layers is a nerve network that controls the movements of the gut. Review Figure 50.13
- Swallowing is a reflex that pushes food into the esophagus. Waves of smooth muscle contraction and relaxation called peristalsis move food from the beginning of the esophagus through the entire length of the gut. Sphincters block the gut at certain locations, but they relax as a wave of peristalsis approaches. Review Figure 50.14
- Enzymatic digestion begins in the mouth, where amylase is secreted with the saliva. Protein digestion begins in the stomach with pepsin and HCl secreted by the stomach mucosa. The mucosa also secretes mucus, protects the tissues of the gut. Review Figure 50.15
- In the duodenum, pancreatic enzymes carry out most of the digestion of the food. Bile from the liver and gallbladder assists in the digestion of fats by breaking them into micelles. Bicarbonate ions from the pancreas neutralize the pH of the chyme entering from the stomach to produce an environment conducive to the actions of pancreatic enzymes. Review Figure 50.16, Table 50.3
- Final enzymatic cleavage of peptides and disaccharides occurs on the surface of the cells of the intestinal mucosa. Amino acids, monosaccharides, and many inorganic ions are absorbed by the microvilli of the mucosal cells. In many cases specific carrier proteins in the membranes of these cells transport nutrients into the cells. Sodium cotransport is a common mechanism for actively absorbing nutrient molecules and ions.
- Fats are absorbed mostly as monoglycerides and fatty acids, which are the product of lipase action on triglycerides in food. These products pass through the membranes of mucosal cells and are then resynthesized into triglycerides within the cells. The triglycerides are combined with cholest-

ANIMAL NUTRITION 909

terol and coated with protein to form chylomicrons, which pass out of the mucosal cells and into lymphatic vessels in the submucosa. Review Figure 50.17

- Water and ions are absorbed in the large intestine so that waste matter is consolidated into feces, which are periodically excreted.
- In herbivores such as rabbits and ruminants, some compartments of the gut have large populations of microorganisms that aid in digesting molecules that otherwise would be indigestible to their host. Review Figure 50.18

Control and Regulation of Digestion

- The processes of digestion are coordinated and controlled by neural and hormonal mechanisms. Salivation and swallowing are autonomic reflexes. Actions of the stomach and small intestine are largely controlled by the hormones gastrin, secretin, and cholecystokinin. Review Figure 50.19

Control and Regulation of Fuel Metabolism

- The liver interconverts fuel molecules and plays a central role in directing their traffic. When food is being absorbed from the gut, the liver takes up and stores fats and carbohydrates, converting monosaccharides to glycogen or fat. The liver also takes up amino acids and uses them to produce blood plasma proteins.
- Fat and cholesterol are shipped out of the liver as low-density lipoproteins. High-density lipoproteins act as acceptors of cholesterol and are believed to bring fat and cholesterol back to the liver.
- Fuel metabolism during the absorptive period is controlled largely by the hormone insulin, which promotes glucose uptake and utilization by most cells of the body, as well as fat synthesis in adipose tissue. During the postabsorptive period, the lack of insulin blocks the uptake and utilization of glucose by most cells of the body except neurons. If blood glucose levels fall, the hormone glucagon is secreted, stimulating the liver to break down glycogen to release glucose to the blood. Review Figures 50.20, 50.21

The Regulation of Food Intake

► Food intake is governed by sensations of hunger and satiety that are determined by brain mechanisms. When one hypothalamic region is damaged, rats eat more and become obese; when another region is damaged, they eat less and

become thin. A number of molecules, such as circulating insulin and glucose, provide feedback information to these brain areas.

► Leptin is a hormone produced by fat cells that inhibits food intake.

Toxic Compounds in Food

► Even natural plant and animal foods can contain toxic compounds in addition to nutrients. Human activities such as the use of pesticides and the release of pollutants into the environment have made the problem of toxins in food even worse.

► An organism can accumulate toxic compounds in its body, especially if those compounds are lipid-soluble or take the structural place of a natural molecule.

► Toxins such as PCBs and DDT that accumulate in the bodies of prey are transferred to and further concentrated in the bodies of their predators. This bioaccumulation produces high concentrations of toxins in animals high up the food chain.

For Discussion

1. Several current popular diet books recommend high fat and protein intake and low carbohydrate intake as a means of losing body mass. What could the rationale of a high-fat and high-protein diet be, and what health issues should be considered when someone considers going on such a diet?

2. Carnivores generally have more dietary vitamin requirements than herbivores do. Why?

3. It is said that the most important hormonal control of fuel metabolism in the postabsorptive period is the lack of insulin. Explain.

4. Why is obstruction of the common bile duct so serious? Consider in your answer the multiple functions of the pancreas and the way in which digestive enzymes are processed.

5. Trace the history of a fatty acid molecule from a piece of buttered toast to a plaque on a coronary artery. What possible forms and structures could it have passed through in the body? Describe a direct and an indirect route it could have taken.

51

Salt and Water Balance and Nitrogen Excretion



Blood, sweat, and tears taste salty because they reflect the composition of the tissue fluid that bathes the cells of the body. The volume and the composition of the tissue fluid must remain within certain limits and must be kept relatively free of wastes. Maintaining homeostasis of the tissue fluid can be challenging. Consider the problems of vampires—not the horror film kind, but the bat kind.

Vampire bats are small, tropical mammals that feed on the blood of other mammals, such as cattle. The bat lands on an unsuspecting (usually sleeping) victim, bites into a vein, and drinks blood—a high-protein, liquid food. The bat has only a short time to feed before the victim wakes and shakes it off. To maximize the volume of blood it can ingest, it eliminates water from its food as fast as it can by producing a lot of very dilute urine. The warm trickle down the neck of the victim is not blood!

Once feeding ends, however, this high rate of water loss cannot continue. Now the vampire bat is digesting protein and must excrete large amounts of nitrogenous breakdown products while conserving its body water. Within minutes, the excretory system of the vampire bat switches from producing lots of very dilute urine to producing a tiny amount of highly concentrated urine. Within minutes, the vampire bat is able to switch from an excretory physiology typical of an animal living in fresh water to an excretory physiology typical of an animal living in an arid desert.

In this chapter we discover how the vampire bat and other species accomplish the various feats of salt and water balance and excretion of wastes that adapt them to many different environments. We begin by discussing the challenges presented by different environments; we use some invertebrate examples to illustrate the basic mechanisms used in the excretory systems of all animals.

Turning to vertebrates, we show that the common anatomical unit that accomplishes all of these tasks is the nephron. The nephron

Blood as a Fast Food

The vampire bat, *Desmodus rotundus*, is able to adjust its excretory physiology from water-excreting to water-conserving, depending on whether it is ingesting or digesting its blood meal.

evolved from a structure that enabled animals living in fresh water to excrete water to a structure that enabled animals living in dry terrestrial habitats to conserve water. Finally, we present the mechanisms that control and regulate salt and water balance in mammals, giving the vampire bat and other species their remarkable abilities to exploit unusual diets and extreme environments.

Tissue Fluids and Water Balance

Life evolved in the seas, and seawater is the extracellular environment for the cells of the simplest marine animals. More complex marine animals have an internal environment consisting of extracellular or tissue fluid, which is isolated from seawater but is similar to it in composition and osmotic concentration. Marine vertebrates and terrestrial animals maintain tissue fluids whose concentration and composition differ considerably from that of seawater (see Chapter 49). The concentration of the tissue fluid determines the water balance of the cells, and its composition influences the health and functions of the cells. Recall, for example, the importance of ionic gradients between the tissue fluid and the cytoplasm of nerve and muscle cells (see Chapter 44).



SALT AND WATER BALANCE AND NITROGEN EXCRETION 911

To understand what is meant by water balance, recall that cell plasma membranes are permeable to water and that the movement of water across membranes depends on differences in solute potential. (See the discussion of osmosis in Chapter 5.) If the solute potential (osmolarity) of the tissue fluid is less negative (that is, the fluid contains fewer solutes) than that of the intracellular fluids, water moves into the cells, causing them to swell and possibly burst. If the solute potential of the tissue fluid is more negative (the fluid contains more solutes) than that of the intracellular fluids, the cells lose water and shrink. The solute potential of tissue fluid determines both the volume and the solute potential of the intracellular environment.

Excretory organs control the solute potential of tissue fluid

Excretory organs control the solute potential and the volume of tissue fluid by excreting solutes that are in excess (such as NaCl when we eat lots of salty food) and conserving solutes that are valuable or in short supply (such as glucose and amino acids). In terrestrial organisms, these excretory organs also eliminate the waste products of nitrogen metabolism. The output of the excretory organs is called urine.

The functions of the excretory organs of a species, and therefore the composition of its urine, depend on the environment in which it lives. We will examine excretory systems that maintain salt and water balance and eliminate nitrogen in marine, freshwater, and terrestrial habitats. In spite of the evolutionary diversity of the anatomical and physiological details, all these systems obey a common rule: there is no active transport of water. Water must be moved either by pressure or by a difference in solute potential. Also, in spite of this evolutionary diversity, there are common mechanisms used by excretory systems.

Mechanisms used by excretory systems include filtration, secretion, and resorption

The excretory systems of many species filter the tissue fluid and then process the filtrate through a system of tubules to produce urine. This filtration process is usually carried out on blood plasma driven across capillary walls in the excretory organ by blood pressure. The filtrate then enters a system of tubules. The cells of the tubules change the composition of the filtrate by active secretion and resorption of specific solute molecules. These three mechanisms are used in the excretory systems of freshwater species, which excrete water and conserve salts, as well as in the systems of marine and terrestrial species, which conserve water and excrete salts.

Distinguishing Environments and Animals in Terms of Salt and Water

The salt concentration, or osmolarity, of ocean water is about 1,070 milliosmoles/liter (mosm/l), and fresh water is generally between 1 and 10 mosm/l. Aquatic environments grade continuously from fresh to extremely salty. Consider

a place where a river enters the sea through a bay or a marsh. Aquatic environments within that bay or marsh range in osmolarity from that of the fresh water of the river to that of the open sea. Evaporating tide pools can reach an even greater osmolarity than seawater. Animals live in all these environments. Some species, called osmoconformers, allow the osmolarity

of their tissue fluids to equilibrate with their environment. Others, called osmoregulators, maintain the osmolarity of their tissue fluids at a constant level as the environment changes.

Most marine invertebrates are osmoconformers

Over a wide range of environmental osmolarities, marine invertebrates simply equilibrate the osmolarity of their tissue fluid with that of the environment. There are limits to osmoconformity, however. No animal could survive if its tissue fluid had the osmolarity of fresh water; nor could animals survive with internal osmolarities as high as those that may be reached in an evaporating tide pool. Such solute concentrations cause proteins to denature.

Osmoregulators regulate the concentration of their tissue fluids

All animals have some solutes in their tissue fluids. Therefore, in fresh water, osmosis will cause water to invade the bodies of animals, so osmoregulation is essential. To osmo-regulate in fresh water, animals must excrete water and conserve solutes; hence they produce large amounts of dilute urine. In salt water, the opposite problem exists. For animals that maintain the osmolarity of their tissue fluids below that of the environment, osmosis will cause a loss of water. To osmoregulate in salt water, animals must conserve water and excrete salts; thus they tend to produce small amounts of very concentrated urine.

Even animals that osmoconform over a wide range of environmental osmolarities must osmoregulate in extreme environments. An excellent example is the brine shrimp *Artemia* (Figure 51.1f), which lives in environments of almost any salinity. *Artemia* are found in huge numbers in the most saline environments known, such as Great Salt Lake in Utah or coastal evaporation ponds where salt is concentrated for commercial purposes (see Figure 26.23). The osmolarity of such water reaches 2,500 mosm/l. At these high environmental osmolarities, *Artemia* is capable of maintaining its tissue fluid osmolarity considerably below that of the environment, and therefore acts as a hypotonic osmoregulator. Very few organisms can survive in the crystallizing brine in which *Artemia* thrives. The main mechanism this small crustacean uses for hypotonic osmoregulation is the active transport of NaCl from its tissue fluids out across its gill membranes to the environment.

Artemia cannot survive in fresh water, but they can live in dilute seawater, in which they maintain the osmolarity of their tissue fluids above that of the environment. Under these conditions, *Artemia* behaves as a hypertonic osmoregulator; that is, it maintains the osmolarity of its tissue fluid above the osmolarity of the environment (Figure 51.1b).

912 CHAPTER FIFTY-ONE



57.7 Environments (") ■ BMHMfWJi&iBieS CO

Can Vary Greatly in Salt

Concentration

(a) Brine shrimp are exposed to a range of very different salinities, (fc>) Animals like the brine shrimp that live at the extremes of environmental osmolarities display flexible osmoregulatory abilities. They become hypertonic osmoregulators in very dilute water, or hypotonic osmoregulators in very saline water.

The composition of tissue fluids can be regulated

Osmoconformers can be ionic conformers, allowing the ionic composition, as well as the osmolarity, of their tissue fluids to match that of the environment. Most osmoconformers, however, are ionic regulators to some degree: They employ active transport mechanisms to maintain specific ions in their tissue fluid at concentrations different from those in the environment.

The terrestrial environment presents problems of salt and water balance that are entirely different from those faced by aquatic organisms. Because the terrestrial environment is extremely desiccating (drying), most terrestrial animals must conserve water. (Exceptions are animals such as muskrats and beavers that spend most of their time in water.)

Terrestrial animals obtain their salts from their food. But plants generally have low concentrations of sodium, so most herbivores must conserve sodium ions. Some terrestrial herbivores travel long distances to naturally occurring salt licks. By contrast, birds that feed on marine animals must excrete the large excess of sodium they ingest with their food. Their nasal salt glands excrete a concentrated solution of sodium chloride via a duct that empties into the nasal cavity. Birds, such as penguins and seagulls, that have nasal salt glands can be seen frequently sneezing or shaking their heads to get rid of the very salty droplets that form (Figure 51.2).

Excreting Nitrogen

The end products of the metabolism of carbohydrates and fats are water and carbon dioxide, which are not difficult to eliminate. Proteins and nucleic acids, however, contain nitrogen, so their metabolism produces nitrogenous wastes in addition to water and carbon dioxide. The most common nitrogenous waste is ammonia (NH₃), which is highly toxic. Ammonia must

be excreted continuously to prevent its accumulation, or it must be detoxified by conversion into other molecules for excretion. Those molecules are principally urea and uric acid (Figure 51.3).

Aquatic animals excrete ammonia

Continuous excretion of ammonia is relatively simple for aquatic animals. Ammonia diffuses in and is highly soluble

Q A brine shrimp in dilute seawater actively transports ions into its body to keep the osmolarity of its tissue fluids above that of the environment..

Q ...but in seawater it allows the osmolarity of its tissue fluids to equilibrate with the environment.

CO

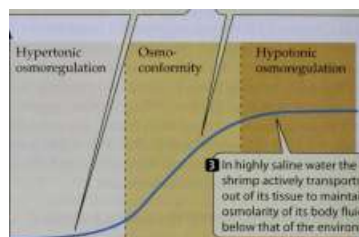
"2 "3

T3 O

c o

Oh

Hypertonic osmoregulation



Dilute seawater

In highly saline water the brine shrimp actively transports ions out of its tissue to maintain the osmolarity of its body fluids below that of the environment.

Seawater

Evaporating salt pond

Osmolarity of environment

in water. Animals that breathe water continuously lose ammonia from their blood to the environment by diffusion across their gill membranes. Animals, such as aquatic invertebrates and bony fishes, that excrete ammonia are said to be ammonotelic.

Many terrestrial animals and some fishes excrete urea

Ammonia is a dangerous metabolite for terrestrial animals that have limited access to water. In mammals, ammonia is lethal when it reaches only 5 mg/100 ml of blood. Therefore, terrestrial (and some aquatic) animals convert ammonia into either urea or uric acid. Ureotelic animals, such as mammals, amphibians, and cartilaginous fishes (sharks and rays), excrete urea as their principal nitrogenous waste product.

Urea is quite soluble in water, but excretion of urea solutions at low concentrations could result in a large loss of water that many terrestrial animals can ill afford. As we will see later in this chapter, mammals have evolved excretory systems that can conserve water by producing concentrated urea solutions. The cartilaginous fishes are another story. These marine species keep their body fluids almost isotonic to the marine environment by retaining high concentrations of urea.

Some terrestrial animals excrete uric acid

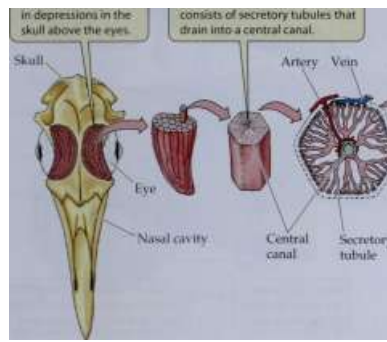
Animals that conserve water by excreting nitrogenous wastes as uric acid are said to be uricotelic. Insects, reptiles, birds, and some amphibians are uricotelic. Uric acid is very insoluble in water and is excreted as a semisolid (for example, the whitish material in bird droppings). Therefore, a uricotelic animal loses very little water as it disposes of its nitrogenous wastes.

SALT AND WATER BALANCE AND NITROGEN EXCRETION 913

Q Salt glands are located in depressions in the

The functional unit of a salt gland consists of secretory tubules that drain into a central canal.

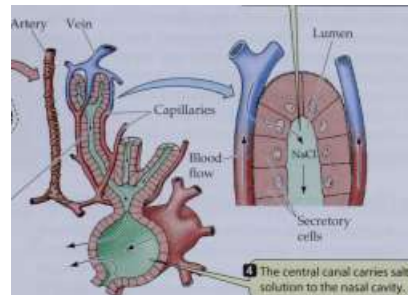
Artery Vein Artery Vein



Na⁺ ions are carried from the blood to secretory tubules via active transport. Cl⁻ ions follow.

^

Secretory tubule



5 7.2 Nasal Salt Glands Excrete Excess Salt

- (a) Marine birds have nasal salt glands adapted to excrete the excess salt from the seawater they consume with their food,
 (b) This giant petrel has returned from a feeding trip at sea and is excreting salt through its nasal salt gland. Note the drop of excreted salt at the tip of the bird's beak.

Most species produce more than one nitrogenous waste

Humans are ureotelic, yet we also excrete uric acid and ammonia. The uric acid in human urine comes largely from the metabolism of nucleic acids and caffeine. In the condition known as gout, uric acid levels in the tissue fluid increase and uric acid precipitates in the joints and elsewhere, caus-

The central canal carries salt solution to the nasal cavity.

(b) *Macronectus giganteus*

f



Note the drop of excreted salt at the tip of the bird's beak.

Carbohydrates Fats



Metabolism of carbohydrates and fat yields only carbon dioxide and water.

Nucleic acids

Amino acids

Nitrogenous bases

-G|H₂

groups

Metabolism of proteins and nucleic acids also yields amino groups...

5 7.3 Waste Products of Metabolism

The metabolism of proteins and nucleic acids produces the nitrogenous wastes ammonia, uric acid, and urea. Most aquatic animals, including most fishes, excrete nitrogenous waste as ammonia. Most terrestrial animals excrete either urea or uric acid. Urea is more soluble in water and is the major N excretory product for mammals and amphibians, as well as some fishes. Uric acid is not very soluble in water and is the major N excretory product for birds, reptiles, and insects.



► oH 3 Ammonia

Ammonotelic animals (aquatic invertebrates and most bony fishes)



Ureotelic animals (mammals,

amphibians, sharks,

cartilaginous fishes)



O

H

Hgr ^c a

|| C=O Uric acid

H

Uricotelic animals (birds, insects, reptiles)

.. .that are excreted in nitrogenous wastes.

914 CHAPTER FIFTY-ONE

ing swelling and pain. The excretion of ammonia is an important mechanism for regulating the pH of the tissue fluid. In some species, different developmental forms live in quite different habitats and have different forms of nitrogen excretion. The tadpoles of frogs and toads, for example, excrete ammonia across their gill membranes, but when they develop into adult frogs or toads, they gener-ally excrete urea. Some adult frogs and toads that live in arid habitats excrete uric acid. These examples show the considerable evolutionary flexibility in how nitrogenous \ a-^tes are excreted.

The Diverse Excretory Systems of Invertebrates

Most marine invertebrates are osmoconformers and have few adaptations for salt and water balance other than active transport mechanisms for ionic regulation. To excrete nitrogen, they can passively lose ammonia by diffusion to the seawater. Freshwater and terrestrial invertebrates, however, have a wide variety of adaptations for maintaining salt and water balance and excreting nitrogen. All of these adaptations are based on the same set of mechanisms: filtration of body fluids and active secretion and resorption of specific ions.

Protonephridia excrete water and conserve salts

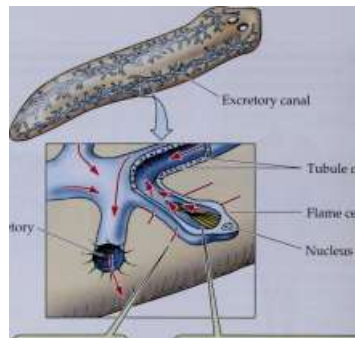
Many flatworms, such as Planaria, live in fresh water. These animals excrete water through an elaborate network of tubules running throughout their bodies. The tubules end in flame cells, so called because each tubule has a tuft of cilia beating inside it, giving the appearance of a flickering flame (Figure 51.4). A flame cell and a tubule together form a protonephridium (plural protonephridia; from the Greek proto, "before," and nephros, "kidney").

Tissue fluid enters the tubules (how it does so is not entirely clear), and the beating of the cilia causes this fluid to flow through the tubules toward the animal's excretory pore. As it flows, the cells of the tubules modify the fluid. As the modified tubule fluid (urine) leaves the planarian, it is less concentrated than the animal's tissue fluid, so ions are conserved and water is excreted by the protonephridium.

Metanephridia process coelomic fluid

Filtration of body fluids and modification of urine by tubules are highly developed processes in annelid worms, such as the earthworm. Recall that annelids are segmented and have a fluid-filled body cavity, called a coelom, in each segment (see Figure 31.23). Annelids have a closed circulatory system through which blood is pumped under pressure (see Figure 49.3). The pressure causes the blood to be filtered across the thin, permeable capillary walls into the coelom. This process is called filtration because the cells and large protein molecules of the blood stay behind in the capillaries while water and small molecules leave them and enter the coelom. In addition, some waste products, such as ammonia, diffuse directly from the tissues into the coelom. But where does this coelomic fluid go?

Excretory • pore



Tubule cells

Body fluids enter the space enclosed by the flame cell...

...and are driven down the tubules toward the excretory pore by the beating of the cilia in the flame cell.

57.4 Protonephridia in Flatworms

The protonephridia of the flatworm *Planaria* consist of tubules ending in flame cells. The tubule cells modify the composition of the fluid passing through them.

Each segment of the earthworm contains a pair of metanephridia (singular metanephridium; from the Greek meta, akin to, and nephros, kidney). Each metanephridium begins in one segment as a ciliated, funnel-like opening in the coelom called a nephrostome, which leads into a tubule in the next segment. The tubule ends in a pore called the nephridiopore, which opens to the outside of the animal (Figure 51.5). Coelomic fluid enters the metanephridia through the nephrostomes. As the fluid passes through the tubules, the cells of the tubules actively resorb certain molecules from it and actively secrete other molecules into it. What leaves the animal through the nephridiopores is a hypotonic (dilute) urine containing nitrogenous wastes, among other solutes.

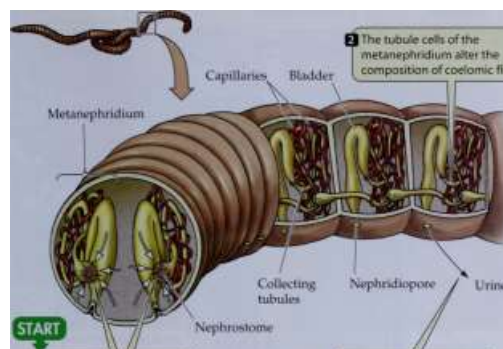
Malpighian tubules are the excretory organs of insects

Insects can excrete nitrogenous wastes with very little loss of water. Therefore, some species can live in the driest habitats on Earth. The insect excretory system consists of blind tubules called Malpighian tubules. An individual insect has from 2 to more than 100 of these tubules attached to the gut between the midgut and hindgut and projecting into the spaces containing tissue fluid (recall that insects have open circulatory systems) (Figure 51.6).

The cells of the Malpighian tubules actively transport uric acid, potassium ions, and sodium ions from tissue fluid into the tubules. As these solutes are secreted into the tubules, water follows because of the difference in solute potential. The walls of the Malpighian tubules have muscle fibers that contract to help move the contents of the tubules toward the hindgut.

H,

I The tubule cells of the metanephridium alter the composition of coelomic fluid...



Urine

Coelomic fluid enters the metanephridium through a nephrostome.

§) ... producing a dilute urine that is excreted through the nephridiopore.



57.5 Metanephridia in Earthworms

The metanephridia of annelids are arranged segmentally. The cross section (left) shows a pair of metanephridia. Longitudinal sections (right) show only one metanephridium of the two in each segment.

The tubule fluid changes in composition while it is in the hindgut. The contents of the hindgut are more acidic than the tubule fluid; as a result, uric acid becomes less soluble and precipitates out of solution as it approaches and enters the rectum. The epithelial cells of the hindgut and rectum actively transport sodium and potassium ions from the gut contents back into the tissue fluid. Because the uric acid molecules have precipitated out of solution, water is free to

follow the resorbed salts back into the tissue fluid through osmosis. Remaining in the rectum are crystals of uric acid mixed with undigested food; this dry matter is what the insect eliminates. The Malpighian tubule system is a highly effective mechanism for excreting nitrogenous wastes and some salts without giving up a significant fraction of the animal's precious water supply.

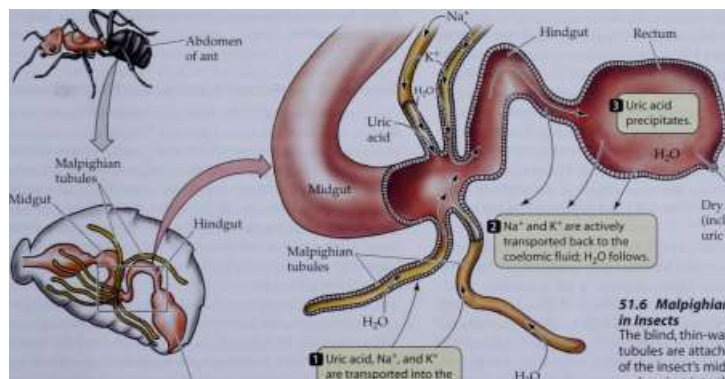
Vertebrate Excretory Systems Are Built of Nephrons

The major excretory organ of vertebrates is the kidney. The functional unit of the kidney is the nephron. Each human kidney has about a million nephrons. All vertebrate kidneys consist of nephrons, yet the kidneys of different species can serve opposite functions to maintain water and salt balance. The kidneys of freshwater fishes, for example, excrete water, but the kidneys of most mammals conserve water.

To understand how the kidney can fulfill opposite functions in different animals, we need to understand how the different parts of the nephron work and the different ways in which they can work together to influence the composition of the urine. The nephron has three main parts:

- ▶ A ball of capillaries called the glomerulus that filters the plasma
- ▶ Renal tubules that receive and modify the filtrate
- ▶ Peritubular capillaries that serve the tubules

Rectum



Rectum

Uric acid, Na^+ , and K^+ are transported into the tubules; H_2O follows.

Dry wastes (including uric acid)

57.6 Malpighian Tubules in Insects

The blind, thin-walled Malpighian tubules are attached to the junction of the insect's midgut and hindgut and project into the spaces containing tissue fluid.

916 CHAPTER FIFTY-ONE

An afferent arteriole supplies blood to the glomerulus.

Q The glomerulus, a knot of capillaries, is the site of blood filtration.

Site of

filtration

(glomerulus)

Site of

tubular

secretion

and

absorption

Urine processing

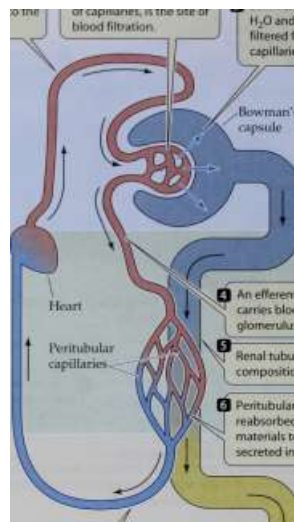
Blood is filtered in the glomerulus

Each nephron has both vascular and tubule components (Figure 51.7). The vascular component is unusual in that it consists of two capillary beds that lie between the arteriole that supplies it and the venule that drains it. The first capillary bed is a dense knot of very permeable vessels called the glomerulus (plural glomeruli) (Figure 51.7). Blood enters the glomerulus through an afferent arteriole and exits through an efferent arteriole. The efferent arteriole gives rise to the second set of capillaries, the peritubular capillaries, which surround the tubule component of the nephron (see Figure 51.7).

The tubule component of the nephron, called a renal tubule, begins with Bowman's capsule, which encloses the glomerulus. The glomerulus appears to be pushed into Bowman's capsule much like a fist pushed into an inflated balloon. Together, the glomerulus and its surrounding Bowman's capsule are called the renal corpuscle. The cells of the capsule that come into direct contact with the glomerular capillaries are called podocytes (see Figure 51.7). These highly specialized cells have numerous armlike extensions, each with hundreds of fine, fingerlike projections. The podocytes wrap around the capillaries so that their fingerlike projections interdigitate and cover the capillaries completely (Figure 51.8b).

The glomerulus filters the blood to produce a tubule fluid that lacks cells and large molecules. The walls of the capillaries, the basal lamina of the capillary endothelium, and the podocytes of Bowman's capsule all participate in filtration. The endothelial walls of the capillaries have pores that allow water and small molecules to leave, but are too small to permit red blood cells to pass through. The mesh-work of the basal lamina is even finer than the pores between the endothelial cells, and it prevents large molecules from leaving the capillaries. Also smaller than the pores in the capillaries are the narrow slits between the fingerlike projections of the podocytes. As a result of these anatomical adaptations, water and small molecules pass from the capillary blood and enter the renal tubule of the nephron (Figure 51.8c), but red blood cells and proteins remain in the capillaries.

The force that drives filtration in the glomerulus is the pressure of the arterial blood. As in every other capillary bed, the pressure of the blood entering the permeable capillaries causes the filtration of water and small molecules. The glomerular filtration rate is high because glomerular capillary blood pressure is unusually high, and because the capillaries of the glomerulus, along with their covering of podocytes, are much more permeable than other capillary beds in the body.



Bowman's capsule receives H₂O and small molecules filtered from glomerular capillaries.

An efferent arteriole carries blood from the glomerulus.

Renal tubule cells alter composition of urine.

Peritubular capillaries carry away reabsorbed substances and bring materials to the tubules that will be secreted into the urine.

T

The renal venule drains the peritubular capillaries.

The processed filtrate (urine) of the individual nephrons enters collecting ducts and is delivered to a common duct leaving the kidney.



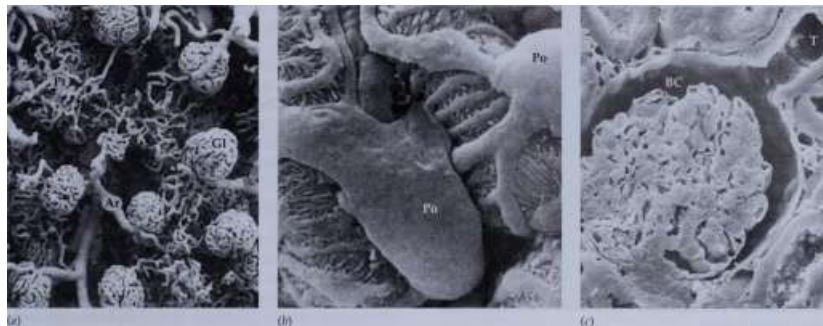
51.7 The Vertebrate Nephron

The vertebrate nephron consists of a renal tubule closely associated with a system of blood vessels. The end of the renal tubule system envelops the glomerulus so that the filtrate from the glomerular capillaries enters the tubules. The tubules change the composition of the filtrate by active absorption and secretion of solutes.

The renal tubules convert glomerular filtrate to urine

The composition of the filtrate that enters the nephron is similar to that of the blood plasma. This filtrate contains glucose, amino acids, ions, and nitrogenous wastes in the same concentrations as in the blood plasma, but it lacks the plasma proteins. As this fluid passes down the renal tubule, its composition changes as the cells of the tubule actively resorb certain molecules from the tubule fluid and secrete other molecules into it. When the tubule fluid leaves the kidney as urine, its composition is very different from that of the original filtrate.

The function of the renal tubules is to control the composition of the urine by actively secreting and resorbing specific molecules. The peritubular capillaries serve the needs of the renal tubules by bringing to them the molecules to be secreted into the tubules and carrying away the molecules that are resorbed from the tubules.



57.8 An SEM Tour of the Nephron

These scanning electron micrographs show the anatomical bases for kidney function. (a) The blood vessels in the kidney, showing the knots of capillaries that form the glomeruli. Each glomerulus (Gl) has an afferent and an efferent arteriole (Ar). Peritubular capillaries (Pt) are looser networks surrounding the tubules of the nephron, (b) Those cells that are in direct contact with the capillaries are the podocytes (Po). Each podocyte has hundreds of tiny fingerlike projections that create filtration slits between them. Anything passing from the glomerular capillaries into the tubule of the nephron must pass through these slits, (c) A cross section of a glomerulus shows that it is surrounded by the tubule cells that form Bowman's capsule (BC), which collects the filtrate and funnels it into the tubule (T) of the nephron. The relationship between the glomerulus and Bowman's capsule is like that of a fist punched into a balloon. Therefore, some of the renal tubule cells are in direct contact with the glomerular capillaries.

Both marine and terrestrial vertebrates must conserve water

Since the vertebrate nephron evolved as a structure for excreting water while conserving salts and essential small molecules, how have vertebrates adapted to environments where water must be conserved and salts excreted? The answer to this question differs for each vertebrate group. Even among the marine fishes, the adaptations of bony fishes are different from those of cartilaginous fishes.

marine bony fishes. Marine bony fishes cannot produce urine more concentrated than their tissue fluid, but unlike most marine animals, they osmoregulate their tissue fluid to only one-fourth to one-third the solute potential of sea-water. They prevent excessive loss of water by producing very little urine. Their urine production is low because their kidneys have fewer glomeruli than do the kidneys of freshwater fishes. In some species of marine bony fishes, the kidneys have no glomeruli at all. Even though the glomeruli are reduced or absent, renal tubules with closed ends are retained for active excretion of ions and certain molecules. Marine bony fishes take in seawater with their food, which results in a large salt load. The fish handle these salt loads by simply not absorbing some ions (such as Mg^{2+} or

So $4 \sim$) from their guts and by actively excreting others (such as Na^+ and Cl^-) from the gill membranes and from the renal tubules. Nitrogenous wastes are lost as ammonia from the gill membranes.

cartilaginous fishes. Cartilaginous fishes are osmocon-formers, but not ionic conformers. Unlike marine bony fishes, cartilaginous fishes convert nitrogenous wastes to urea and another compound called tri-methyl amine oxide and retain large amounts of these compounds in their tissue fluids. As a result, their tissue fluids have an osmolarity close to that of seawater. These species have adapted to a concentration of urea in the body fluids that would be fatal to other vertebrates.

Sharks and rays still have the problem of excreting the large amounts of salts they take in with their food. They have several sites of active secretion of $NaCl$, but the major one is a salt-secreting rectal gland.

amphibians. Most amphibians live in or near fresh water and stay in humid habitats when they venture from the water. Like freshwater fishes, most amphibian species produce large amounts of dilute urine and conserve salts. Some amphibians, however, have adapted to habitats that require water conservation.

918 CHAPTER FIFTY-ONE



Lymnodynastes dumerilii

51.9 Burrowing Frogs

The banjo frog of the Australian desert survives long droughts by burrowing deep in the sand and entering estivation, a state of low metabolic activity. These frogs store water in the form of dilute urine in their enormous bladders.

Amphibians living in very dry terrestrial environments have reduced the water permeability of their skin. Some secrete a waxy substance that they spread over the skin to waterproof it. Several species of frogs that live in arid regions of Australia burrow deep into the ground, where they remain during long dry periods (Figure 51.9). There they enter estivation, a state of very low metabolic activity and therefore low water turnover. When it rains, these frogs come out of estivation, feed, and reproduce. But their most interesting adaptation is that they have enormous urinary bladders. Before entering estivation, they fill their bladders with dilute urine, which can amount to one-third of their body weight. This dilute urine serves as a water reservoir that they use gradually during the long period of estivation. Australian aboriginal peoples dig up estivating frogs as an emergency source of drinking water.

Third, they excrete nitrogenous wastes as uric acid solids, therefore losing little water in the process.

birds. Birds have the same adaptations for water conservation that reptiles have: internal fertilization, shelled eggs, skin that retards water loss, and uric acid as the nitrogenous waste product. In addition, some birds can produce a urine that is more concentrated than their tissue fluids. This last ability is most developed in mammals.

The Mammalian Excretory System

The adaptations of mammals and birds for producing urine hypertonic to their tissue fluids were an important step in vertebrate evolution. These adaptations enabled the excretory system to conserve water while still excreting excess salts and nitrogenous wastes. Mammals and birds have high body temperatures and high metabolic rates, and therefore have the potential for a high rate of water loss. Being able to minimize water loss from their excretory systems made it possible for these highly active species to occupy arid habitats.

We have seen how the nephron originally evolved to excrete water; now we will see how it can serve as the basic structural unit of an organ that is able to conserve water. To understand this evolutionary change of function, it is necessary to understand the structure and function of the nephron in the context of the overall anatomy of the kidney. First, however, let's look at the mammalian excretory system as a whole.

Kidneys produce urine, which the bladder stores

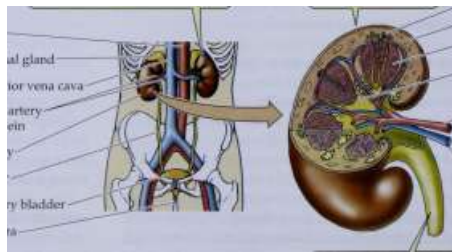
We will use humans here as our example of the mammalian excretory system. Humans have two kidneys just under the dorsal wall of the abdominal cavity in the mid-back region (Figure 51.10). Each kidney filters blood, processes the fil-

The kidneys are positioned in the upper rear of the abdominal cavity.

The internal structure of the kidney includes a cortex and, beneath it, a medulla.

Aorta -

reptiles. Reptiles occupy habitats ranging from aquatic to extremely hot and dry. Three major adaptations have freed the reptiles from maintaining the close association with water that is necessary for most amphibians. First, reptiles do not need fresh water to reproduce, because they employ internal fertilization and lay eggs with shells that retard evaporative water loss. Second, they have scaly, dry skins that retard evaporative water loss.



Adrenal gland— Posterior vena cava

Renal artery and vein

Kidney Ureter

Urinary bladder Urethra

5.7 The Human Excretory System

The human kidney has a regular internal tissue structure that is the basis for its function of filtering the blood and producing urine.

Nephron

Cortex

Medulla

Renal pyramid

Renal artery

Renal vein



Urine leaves the kidney from the inner surface of the medulla and is collected in the ureter.

Urine enters the urinary bladder, where the urine is stored until it is excreted through the urethra. The urethra is a short tube that opens to the outside at the end of the penis in males or just anterior to the vaginal opening in females.

Two sphincter muscles surrounding the base of the urethra control the timing of urination. One of these sphincters is a smooth muscle and is controlled by the autonomic nervous system. When the bladder is full, a spinal reflex relaxes this sphincter. This reflex is the only control of urination in infants, but the reflex gradually comes under the influence of higher centers in the nervous system as a child grows older. The other sphincter is a skeletal muscle and is controlled by the voluntary, or conscious, nervous system. When the bladder is very full, only serious concentration prevents urination.

Nephrons have a regular arrangement in the kidney.

The kidney is shaped like a kidney bean; when cut down its long axis and split open as a bean splits open, its important anatomical features are revealed (see Figure 51.10). The ureter and the renal artery and renal vein enter the kidney on its concave (punched-in) side. The ureter divides into several branches, the ends of which envelop kidney tissues called renal pyramids. The renal pyramids make up the internal core, or medulla, of the kidney. The medulla is surrounded by tissue with a different appearance, called the cortex. The renal artery and vein give rise to many arterioles and venules in the region between the cortex and the medulla. Each human kidney contains about a million nephrons, and their organization within the kidney is very regular. All of the glomeruli are located in the cortex. The initial segment of a renal tubule is called the proximal convoluted tubule— "proximal" because it is close to its glomerulus and "convoluted" because it is twisted (Figure 51.11). All the proximal convoluted tubules are also located in the cortex.

At a certain point, the renal tubule takes a dive directly down into the medulla. The portion of the tubule in the medulla is called the loop of Henle. It is called a loop because it runs straight down into the medulla, makes a hairpin turn, and comes straight back to the cortex. Where the ascending limb of the loop of Henle reaches the cortex, it becomes the distal convoluted tubule— "distal" because it is farther from its glomerulus than the proximal tubule is. The distal convoluted tubules of many nephrons join a common collecting duct in the cortex. The collecting ducts then run in parallel with the loops of Henle down through the medulla and empty into the ureter at the tips of the renal pyramids.

Blood vessels also have a regular arrangement in the kidney.

The organization of the blood vessels of the kidney closely parallels the organization of the nephrons (see Figure 51.11). Arterioles branch from the renal artery and radiate into the cortex. An afferent arteriole carries blood to each

The glomeruli, the proximal convoluted tubules, and the distal convoluted tubules are in the cortex.

Afferent arteriole

The loops of Henle and the vasa recta are in the medulla.

Proximal

convoluted

tubule

Distal

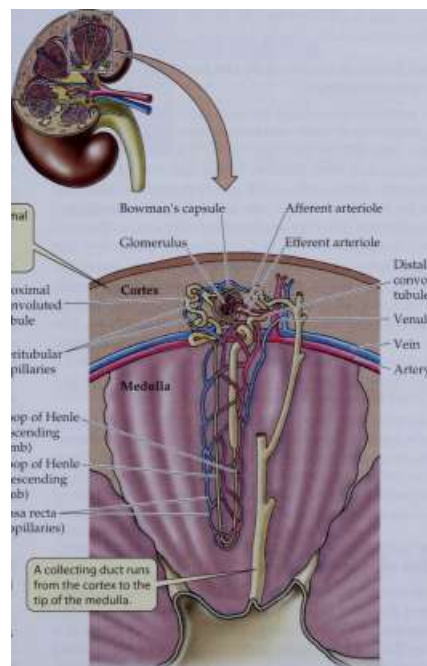
convoluted

tubule

Outline of renal pyramid

57.77 The Organization of the Nephron within the Mammalian Kidney

The glomeruli and major portions of the renal tubules are located in the cortex of the kidney, but portions of the renal tubules, called the loops of Henle, run in parallel as straight sections down into the renal medulla and back up to the cortex. Collecting ducts run from the cortex to the inner surface of the medulla, where they open into the ureter.



Peritubular capillaries

Loop of Henle

(ascending

limb)

Loop of Henle

(descending

limb)

Vasa recta

(capillaries)

A collecting < from the cortex tip of the medu

920 CHAPTER FIFTY-ONE

erulus. Drain nerulu> teri-

ole that . es se to the peritubular capillaries, mo-which surround th 1 portions of the tubules.

A few peritubular capil the medulla in

the loops of Henle and the collecting duct

These capillaries form the vasa recta. All the peritubular

capillaries, a nephron collect into a venule

that joins with venules from other nephrons eventually

the renal vein, which takes blood from the kidney

The volume of glomerular filtration is greater than the volume of urine

excreted and solutes filtered in the glomeruli. ~

appears in the urine. We can reach this by comparing the rate of filtration by the glomeruli with the rate of urine production. The kidney

filters 1 liter of blood per minute, or more than

1.5 L of blood per day. How much of this huge volume is filtered in the glomeruli? The answer is about 12

liters. This is still a large volume. ~ 120 L per day

Since normally only 1 L of urine is excreted per day, about 1%

of the fluid volume that is filtered in the glomerulus is excreted into the blood. Where and how is this enormous fluid volume resorbed?

Most filtrate is resorbed by the proximal convoluted tubule

The proximal convoluted tubule is responsible for most of the resorption of water and solutes from the glomerular filtrate.

The cells of this section of the renal tubule are cuboidal, and their surface facing the tubule has thousands of microvilli, which greatly increase their surface area for resorption. These cells have lots of mitochondria—an indication that they are biochemically active. The tubule actively transports water and other solutes, such as glucose and amino acids: the

tubule fluid. Almost all glucose and amino acid molecules that are filtered from the blood are actively resorbed by these cells and transported back into the tissue fluid. The active transport of solutes into the tissue fluid causes water to follow osmotically. The water and solutes moved into the tissue fluid are taken up by the peritubular capillaries and returned to the venous blood leaving the kidney.

Despite the large volume of water and solutes resorbed

in the proximal convoluted tubule, the overall concentration of solutes in the tubule fluid is not different from that of the blood plasma, although its composition is quite different. How, then, does the kidney produce urine that is more concentrated than the blood plasma?

The loop of Henle creates a concentration gradient in the surrounding tissue

Humans can produce urine that is four times more concentrated than their blood plasma. The vampire bat we encountered at the beginning of this chapter can produce

urine that is 10 times more concentrated than its blood. Some desert-dwelling animals

are able to produce urine that is even more concentrated. The ability of the mammalian kidney is due to the loops of

Henle, which function as a countercurrent multiplier system. The term "countercurrent" refers to the fact that tubule fluid in the descending limb of the loop flows in the opposite direction from that in the ascending limb. "Multiplier" refers to the ability of this system to create a concentration gradient in the renal medulla. The loops of Henle do not themselves produce a concentrated urine; rather, they increase the solute potential of the surrounding tissue fluid.

The segments of the loop of Henle differ anatomically and functionally. Cells of the descending limb and the initial cells of the ascending limb are flat, with no microvilli and few mitochondria. They are not specialized for transport. Partway up the ascending limb, the cells become specialized for active transport. They are cuboidal and have

lots of mitochondria. Accordingly, the loop of Henle is divided into the thin descending limb, the thin ascending limb, and the thick ascending limb. To understand the countercurrent multiplier mechanism, it is easiest to move backward through the renal tubule, starting with the thick ascending limb (Figure 51.11).

The thick ascending limb actively resorbs Na⁺ (with Na⁺-K⁺-ATPase)

from the tubule fluid and moves it into the

surrounding tissue fluid. The thick ascending limb is not permeable

meafc -. : atei 90 the resorption of Na^+ and Cl^- raises the concentration of these solutes in the surrounding tissue fluid.

The thin descending limb, in contrast, is rather permeable to water, but not very permeable to Na^+ and Cl^- . Since surrounding tissue fluid has been made more concentrated by the Na^+ and Cl^- resorbed from the neighboring thick ascending limb, water is withdrawn osmotically from the tubule fluid in the descending limb. Therefore, the fluid in the descending limb becomes more and more concentrated as it flows toward the bottom of the renal medulla.

The thin ascending limb, like the thick ascending limb, is not permeable to water. It is, however, permeable to Na^+ and Cl^- . As the concentrated tubule fluid flows up the thin ascending limb, it is more concentrated than the surrounding tissue fluid, so Na^+ and Cl^- diffuse out of it. When the tubule fluid reaches the thick ascending limb, active transporters move Na^+ and Cl^- from the tubule fluid to the tissue fluid as we saw above.

As a result of the processes described above, the tubule fluid reaching the distal convoluted tubule is less concentrated than the blood plasma, and the solutes that have been left behind in the renal medulla have created a concentration gradient in the surrounding tissue fluid. The tissue fluid in the renal medulla becomes more and more concentrated as we move from the border with the cortex to the tips of the renal pyramids.

Urine is concentrated in the collecting ducts

As Na^+ and Cl^- are transported out of the tubule fluid, urea and other waste products make up a greater proportion of its total solute content as it flows toward the collecting duct. Therefore, the tubule fluid entering the collecting duct

is less concentrated than the blood plasma, but its composition is considerably different from that of the plasma.

Cortex

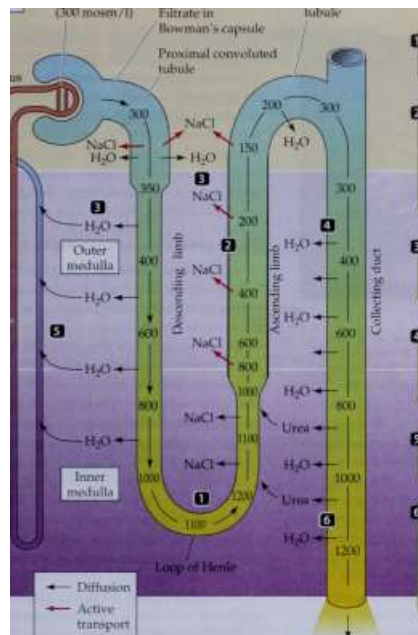
300

300

Glomerulus 300

Blood plasma (300 mosm/l)

Distal convoluted tubule



Q The loop of Henle acts as a countercurrent multiplier to establish a concentration gradient in the renal medulla.

Q The thick segment of the ascending limb

pumps NaCl out of the urine and into the tissue fluid, but H_2O cannot follow, because this region of the tubule is impermeable to water. Continued pumping of NaCl from the thick ascending limb sets up a concentration gradient in the renal medulla.

Increased concentration of NaCl in the tissue fluid causes osmotic absorption of water from the descending limb, thus concentrating the tubule fluid that enters the ascending limb.

The urine entering the collecting duct is less concentrated than the tissue fluid, so as urine passes down the collecting duct it loses water to the tissue fluid and becomes more and more concentrated.

o Water resorbed from the descending limb and the collecting duct leaves the medulla in the vasa recta.

fj The lower collecting duct is permeable to urea as well as to water. Urea is very concentrated in the urine at this point, so it diffuses into the tissue fluid. The increased osmolarity of the tissue fluid enhances the countercurrent multiplier effectiveness. Urea enters the ascending limb and is recycled.



H⁵ 7.72 Concentrating the Urine

The countercurrent multiplier mechanism enables the kidney to produce urine that is far more concentrated than mammalian blood plasma.

The tubule fluid entering the distal convoluted tubule loses water osmotically as it flows toward the collecting duct.

The concentration gradient established in the renal medulla by the loops of Henle enables the urine to be concentrated in the collecting ducts. The collecting ducts begin in the renal cortex and run through the renal medulla before emptying into the ureter at the tips of the renal pyramids. As the solute concentration of the surrounding tissue fluid increases, more and more water is absorbed from the urine in the collecting duct. By the time it reaches the ureter the urine has been greatly concentrated.

It follows from the process we have just described that the ability of a mammal to concentrate its urine will be determined by the maximum concentration gradient it can establish in its renal medulla. One way to increase the con-

J

centration gradient is to increase the lengths of the loops of Henle. That is precisely the adaptation we find in mammals that live in extremely arid habitats. The desert gerbil, for example, has such extremely long loops of Henle that its renal pyramids (each of its kidneys has only one, in contrast to

Urine

ours) extends far out of the concave surface of the kidney and into the ureter (Figure 51.13). These animals are so effective in conserving water that they can survive on the water released by the metabolism of their dry food; they do not need to drink!

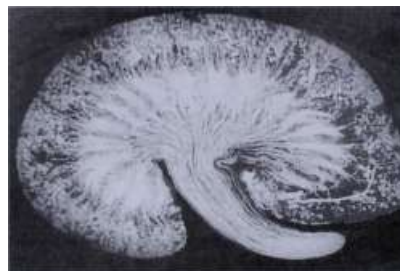
Control and Regulation of Kidney Functions

Control and regulatory mechanisms act on the kidneys to maintain blood osmolarity and blood pressure. We will discuss these various mechanisms separately, but keep in mind that they are always working together.

The kidneys act to maintain the glomerular filtration rate

If the kidneys stop filtering blood, they cannot accomplish any of their functions. The glomerular filtration rate (GFR) depends on an adequate blood supply to the kidneys at an adequate blood pressure. Therefore, the kidneys have mechanisms to maintain their blood supply and blood pressure regardless of what is happening elsewhere in the body. Because these adaptations of the kidney support the maintenance of kidney function, they are called autoregula-

922 CHAPTER FIFTY-ONE



57.73 The Ability to Concentrate

The ability of the mammalian kidney to concentrate urine depends on the lengths of its loops of Henle relative to the overall size of the kidney. Some desert rodents have single renal pyramids so long that they protrude out of the kidney and into the ureter.

tory mechanisms. The kidney's autoregulatory adjustments compensate for decreases in cardiac output or decreases in blood pressure so that the GFR remains high (Figure 51.14).

One autoregulatory mechanism is the dilation (expansion) of the afferent renal arterioles when blood pressure falls. This dilation decreases the resistance in the arterioles and helps maintain blood pressure in the glomerular capillaries. If arteriole dilation does not keep the GFR from falling, then the kidney releases an enzyme, renin, into the blood. Renin acts on a circulating protein to begin converting it into an active hormone called angiotensin.

Angiotensin has several effects that help restore the GFR to normal. First, angiotensin causes the efferent renal arterioles to

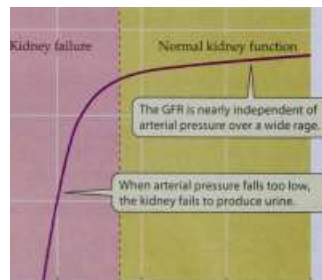
constrict, which elevates blood pressure in the

150 -

S 100

50

'J



50 100 150

Arterial pressure (mm Hg)

200

57.74 Maintaining the Glomerular Filtration Rate

Glomerular filtration is driven by arterial pressure, but autoregulatory mechanisms prevent rises and falls in glomerular filtration rate (GFR) over a wide range of pressures.

glomerular capillaries. Second, it causes peripheral blood vessels all over the body to constrict—an action that elevates central blood pressure. Third, it stimulates the adrenal cortex to release the hormone aldosterone. Aldosterone stimulates sodium resorption by the kidney, thereby making the resorption of water more effective. Enhanced water resorption helps maintain blood volume and therefore central blood pressure. Finally, angiotensin acts on structures in the brain to stimulate thirst. Increased water intake in response to thirst increases blood volume and blood pressure.

Blood pressure and osmolarity are regulated by ADH

When you lose blood volume, your blood pressure tends to fall. Besides activating the kidney autoregulatory mechanisms described in the previous section, a drop in blood pressure decreases the activity of the stretch receptors in the walls of the aorta and the carotid arteries (see Chapter 49). These stretch receptors provide information to cells in the hypothalamus that produce antidiuretic hormone (ADH, also called vasopressin) and send it down their axons to the posterior pituitary gland (see Chapter 41). As stretch receptor activity decreases, the production and release of this hormone increases (Figure 51.15).

ADH acts on the collecting ducts of the kidney to increase their permeability to water. When the circulating level of ADH is high, the collecting ducts are very permeable to water, more water is resorbed from the urine, and only small quantities of concentrated urine are produced, thus conserving blood volume and blood pressure. When ADH levels are low, water is not resorbed from the collecting ducts, and lots of dilute urine is produced.

ADH controls the permeability of the collecting ducts by stimulating the production and activity of membrane proteins that form water channels. These proteins, called aquaporins, are found in many tissues that are permeable to water—for example, the capillary endothelium, red blood cells, and the proximal convoluted tubules of the kidney. Differences among tissues in water permeability can be related to the presence or absence of aquaporins. Aquaporins are expressed in the descending limb of the loop of Henle, for example, but not in the ascending limb. One particular aquaporin is found in collecting duct cells and is controlled by ADH on both a long-term and a short-term basis. Over the long term, ADH levels influence the expression of the gene for this aquaporin; over the short term, ADH controls the insertion of the aquaporin into the cell membranes.

ADH also helps regulate blood osmolarity. Sensory cells in the hypothalamus monitor the solute potential of the blood. If blood osmolarity increases, these osmoreceptors stimulate increased release of ADH to enhance water resorption from the kidneys. The osmoreceptors also stimulate thirst. The resulting water retention and water intake dilutes the blood as it expands blood volume.

The heart produces a hormone that influences kidney function

When blood pressure becomes abnormally high, or when a weakened heart cannot pump blood effectively, the atria of

Regulation of blood osmolarity

Rise in blood

osmolarity

Regulation of blood pressure

Rise in

blood

pressure

Osmoreceptors detect an increase in osmolarity and

stimulate ADH release

t

G

Renal excretion

of solute and

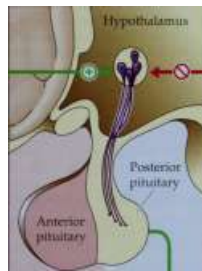
resorption of H_2O

decreases blood

osmolarity

In the kidney: ADH increases permeability of collecting duct cells to H_2O

Hypothalamus



Stretch receptors detect increases in

blood pressure

and inhibit ADH

release

k

Circulating ADH

Constriction of

peripheral blood

vessels elevates

blood pressure

In the periphery:

ADH causes

blood vessels

to constrict

C

Resorption of water helps maintain blood volume

57.75 Antidiuretic Hormone Increases Blood Pressure and Promotes Water Resorption

ADH is produced by neurons in the hypothalamus and released from their axons in the posterior pituitary. The release of ADH is stimulated by hypothalamic osmoreceptors and inhibited by stretch receptors in the great arteries.

the heart become stretched. When the atrial muscle fibers are stretched too much, they release a peptide hormone called atrial natriuretic hormone. This hormone enters the circulation, and when it reaches the kidney, it decreases the resorption of sodium. The result is an increased loss of sodium and water, which has the effect of lowering blood volume and blood pressure.

Q Sodium ions (Na^+) and bicarbonate ions (HCO_3^-) are filtered in the glomerulus.

The kidneys help regulate acid-base balance

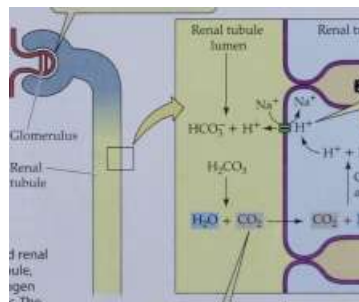
Besides salt and water balance and nitrogen excretion, the kidneys have another important role in regulating the hydrogen ion concentration (the pH) of the blood. pH is a critical variable because it influences the structure and therefore the function of proteins. One way to minimize changes in a chemical solution is to add a buffer—a substance that can either absorb excess hydrogen ions or supply hydrogen ions (see Chapter 2). The major buffer in the blood is the bicarbonate ion, HCO_3^- , which is formed from the disassociation of carbonic acid, which in turn is formed by the hydration of CO_2 according to the following equilibrium reactions (see Chapter 48):

$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$ —You can see that if excess H^+ ions are added to this reaction mix, the reaction will move to the left and absorb the excess H^+ . On the other hand, if H^+ ions are removed from the reaction mix, the reaction will move to the right and supply more H^+ ions.

The bicarbonate buffer "system" is important for controlling the pH of the blood because the reactions can be pushed and pulled physiologically. The lungs control the levels of CO_2 in the blood, and the kidneys control the levels of H^+ and HCO_3^- ions in the blood. The renal tubules secrete H^+ and resorb HCO_3^- (Figure 51.16). The kidney has other buffering systems as well, and together they greatly enhance the ability of the kidney to eliminate acid.

57.76 The Kidney Excretes Acids and Conserves Bases

Bicarbonate ions are filtered in the glomerulus, and renal tubule cells secrete hydrogen ions. In the renal tubule, the filtered bicarbonate buffers the secreted hydrogen ions and keeps the urine from becoming too acidic. The CO_2 formed by the reaction of bicarbonate and hydrogen ions is converted back to bicarbonate by the renal tubule cells and transported back into the tissue fluids.



Renal tubule cells

J

Tissue fluids

Renal tubule cells secrete H^+ in exchange for Na^+ .

$\text{H}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}_2\text{CO}_3$ — A Carbonic anhydrase

$\text{CO}_2 + \text{H}_2\text{O}$

$\rightarrow \text{Na}^+ + \text{HCO}_3^-$

Q CO_2 is formed by the reaction

of HCO_3^- and H^+ and diffuses into tubule cell.

o CO_2 is converted back to

bicarbonate in renal tubule cells and transported back into tissue fluids.

924 CHAPTER FIFTY-ONE

Chapter Summary

Tissue Fluids and Water Balance

► The problems of salt and water balance and nitrogen excretion that animals face depend on their environments, but in all animal excretory systems, there is no active transport of water.

► All adaptations for maintaining salt and water balance and for excreting nitrogen wastes employ the same basic

mechanisms: filtration of body fluids and active secretion and resorption of specific ions.

Distinguishing Environments and Animals in Terms of Salt and Water

► Marine animals can be osmoconformers or osmoregulators. Freshwater animals must be osmoregulators and must continually excrete water and conserve salts. All animals are ionic regulators to some degree. Review Figure 51.1

► On land, water conservation is essential, and diet determines whether salts must be conserved or excreted. Marine birds excrete excess salt through nasal salt glands. Review Figure 51.2

Excreting Nitrogen

► Aquatic animals can eliminate nitrogenous wastes such as ammonia by diffusion across their gill membranes. Terrestrial animals must detoxify ammonia by converting it to urea or uric acid for excretion. Review Figure 51.3

► Depending on the form in which they excrete their nitrogenous waste products, animals are classified as ammonotelic, ureotelic, or uricotelic.

The Diverse Excretory Systems of Invertebrates

► The protonephridia of flatworms consist of flame cells and excretory tubules. Tissue fluid is filtered into the tubules, which process the filtrate to produce a dilute urine. Review Figure 51.4

► In annelid worms, blood pressure causes filtration of the blood across capillary walls. The filtrate enters the coelomic cavity, where it is taken up by open-ended tubules called metanephridia. As the filtrate passes through the tubules to the outside, its composition is changed by active transport mechanisms. Review Figure 51.5

► The Malpighian tubules of insects receive ions and nitrogenous wastes by active transport across the tubule cells. Water follows by osmosis. Ions and water are resorbed from the rectum, so the insect excretes semisolid wastes. Review Figure 51.6

Vertebrate Excretory Systems Are Built of Nephrons

► The nephron, the functional unit of the vertebrate kidney, consists of a glomerulus, in which blood is filtered across the walls of a knot of capillaries, and a renal tubule, which processes the filtrate into urine to be excreted. A system of peritubular capillaries serves the tubule. Review Figures 51.7, 51.8

► The adaptations of marine fishes and terrestrial animals to conserve water are diverse. Bony fishes have few glomeruli and produce little urine. Cartilaginous fishes retain urea so that the osmotic concentration of their body fluids remains above that of seawater. Amphibians remain close to water or have waxy skin coverings. Reptiles have scaly skin, lay shelled eggs, and excrete nitrogenous wastes as uric acid.

► Birds share the adaptations of reptiles; in addition, they can produce urine more concentrated than their tissue fluids. Only birds and mammals can produce such urine.

The Mammalian Excretory System

► The concentrating ability of the mammalian kidney depends on its anatomy. Review Figure 51.10

► The glomeruli and the proximal and distal convoluted tubules are located in the cortex of the kidney. Certain molecules, salts, and water are resorbed in bulk, and other molecules are actively secreted in the convoluted tubules without the urine becoming more concentrated. Straight sections of renal tubules called loops of Henle and collecting ducts are arranged in parallel in the medulla of the kidney. Review Figure 51.11

► The loops of Henle create a concentration gradient in the extracellular fluids of the renal medulla by a countercurrent multiplier mechanism. Urine flowing down the collecting ducts to the ureter is concentrated by the osmotic loss of water caused by the concentration gradient in the surrounding tissue fluid. Review Figure 51.12

Control and Regulation of Kidney Functions

► Kidney function in mammals is controlled by autoregulatory mechanisms that maintain a constant high glomerular filtration rate even if blood pressure varies. Review Figure 51.14

► An important autoregulatory mechanism is the release of renin by the kidney when blood pressure falls. Renin activates angiotensin, which causes the constriction of peripheral blood vessels, causes the release of aldosterone (which enhances water resorption), and stimulates thirst.

► Kidney function in mammals is also controlled by mechanisms responsive to blood pressure and osmolarity. Changes in these variables influence the release of antidiuretic hormone, which controls the permeability of the collecting duct to water and therefore the amount of water that is resorbed from the urine. ADH stimulates the expression of proteins called aquaporins that serve as water channels in the membranes of collecting duct cells. Review Figure 51.15

► Hydrogen ions secreted by renal tubules are buffered in the urine by bicarbonate and other buffering systems. Review Figure 51.16

For Discussion

1. Why is it said that the oceans are a physiological desert? For what animals would this apply?
2. Persons with uncontrolled diabetes mellitus can have very high levels of glucose in their blood. Why do such individuals have a high level of urine production?
3. Inulin is a molecule that is filtered in the glomerulus, but is not secreted or resorbed by the renal tubules. If you injected inulin into an animal and after a brief time measured the concentration of inulin in its blood and urine, how could you determine the animal's glomerular filtration rate? Assume that the rate of urine production is 1 ml per minute.
4. After you did the inulin experiment to measure glomerular filtration rate, how could you use that information to determine whether another substance is secreted or resorbed by the renal tubules? Assume you can measure the concentration of that substance in the blood and in the urine. Urine production is still 1 ml per minute.
5. Explain what would happen with respect to control and regulation of your salt and water balance if you went to a movie and ate a lot of very salty popcorn.



Animal Behavior

*

A troop of Japanese macaques living on an island was being studied by scientists, who fed the monkeys by throwing pieces of sweet potatoes onto the beach from a passing boat. The monkeys tried to brush the sand off the sweet potatoes, but they were still gritty. One day a young female monkey began taking her sweet potatoes to the water and washing them. Soon her siblings and other juveniles in her play group imitated her new behavior. Next their mothers began washing their potatoes. No adult males imitated the behavior of the juveniles or the adult females, but young males learned the behavior from their mothers and their siblings.

The scientists were fascinated by the way the creative, insightful behavior of one juvenile female spread through the population, so they presented the monkeys with a new challenge: They threw wheat onto the beach. Picking grains of wheat out of the sand was tedious and difficult. The same juvenile female came up with a solution: She carried handfuls of sand and grain to the water and threw them in. The sand sank, and the grain floated, enabling her to skim it off the surface and eat it. This behavior spread through the population in the same way potato washing did, first to other juveniles, then to mothers, and then from mothers to both their male and female offspring.

The macaques now routinely wash their food. They play in the water, which they did not do before, and they have added some marine items to their diet. Clearly, this population of monkeys has invented new behaviors that have spread by imitative learning and have become traditions in the population. One could say that they have acquired a culture: a set of behaviors shared by the population and transmitted by learned traditions.

The reason this study of macaques is so interesting is that it erodes what seemed to

Learned Behaviors Shared by a Population Become a Culture

In the space of only a few generations, a population of Japanese macaques (*Macaco fuscata*) learned and transmitted a set of behaviors that included washing food, playing in the water, and eating marine food items—a new "culture" of water-related behaviors.

be a clear distinction between human behavior and the behavior of other animals. The behavior of most animals is largely determined by heredity, with learning playing a relatively minor role. In contrast, most human behaviors are acquired through cultural traditions and learning. The fact that other primates can invent novel behaviors and pass them on culturally shows that there is no absolute dividing line between human and animal behavior.

We begin this chapter with descriptions of some classic studies of behaviors that are largely shaped by inheritance, but to varying degrees are modified by experience. Then we explore how hormones influence the development and expression of behavior.

Next we discuss animal communication, showing how this behavior has been shaped by natural selection. Then we look at studies of biological rhythms and navigation to see how the mechanisms underlying these behaviors have been investigated. Throughout the chapter, we hope you will use what you read to raise your own questions about human behavior, to which we will return at the end of the chapter.



926 CHAPTER FIFTY-TWO

What, How, and Why Questions

Most species of animals can be identified by their behaviors. Behavior is highly visible and shows us what an animal does: how it gets food, how it avoids dangers in the environment, and how it reproduces. Behavior is highly adaptive, and it is therefore not surprising that many behaviors are shaped by natural selection, and are highly species-specific. On the other hand, flexibility of behavior can be extremely valuable to an animal that has to deal with changing conditions and complex situations, as in social interactions. Therefore, to varying degrees, behavior is modifiable by learning.

In studying any behavior, we can ask what, how, and why questions. What questions focus on the details of behavior, including the proximate cause of the behavior—in other words, what stimuli cause the animal to express the behavior. How questions are about the mechanisms of behavior—the underlying neural, hormonal, and anatomical mechanisms that we have been studying in Part Six. How questions can also focus on the means by which an animal acquires a behavior—the relative roles of genetically determined mechanisms and experience. Most behaviors involve complex interactions of inherited anatomical and physiological mechanisms and the ability to alter behavior through learning.

Why questions have to do with the ultimate causes of behavior—the selective pressures that shaped its evolution. In this chapter we will frequently discuss the adaptive nature of behavior, but the evolution of behavior will be the major focus of the next chapter.

Behavior Shaped by Inheritance

Much of the behavior of many animals is highly stereotypic (it is performed in the same way every time) and species-specific (there is little variation in the way different individuals of the same species perform it). We can identify species of spiders, for example, by their web designs (Figure 52.1). Web spinning requires thousands of movements performed in just the right sequence, and for a given species, most of that sequence is performed the same way every time. Different spider species spin webs of different designs, using different sequences of movements.

Web spinning by spiders is also an example of a complex behavior that requires no learning or prior experience. When juvenile spiders hatch, their mother is already dead, and they disperse immediately (remember *Charlotte's Web* by E. B. White?). They have no experience of their mother's web. Yet, when they construct their own webs, they do it perfectly without the benefit of experience or a model to



52.1 Spider Web Designs Are Species-Specific

Each spider performs a stereotypic sequence of movements typical of its species that results in a species-specific web design.

copy. In fact, their web spinning behavior is actually rather resistant to modification by learning. When confronted experimentally with challenges to web construction, young spiders appear incapable of learning how to modify the design of their webs.

Many classic studies of stereotypic and species-specific behaviors were performed by scientists who studied the behavior of animals in nature—a field called ethology. The early ethologists asked to what extent such behaviors are determined by inheritance and to what extent they are modifiable by experience. Two experimental approaches were used to test whether behaviors are hereditary: (1) depriving animals of opportunities to learn and (2) studying the behavior of the offspring of two parents that differ in their behavior.

1. Tail shake i
2. Head flick
3. Tail shake
4. Bill shake
5. Grunt whistle
6. Tail shake



Deprivation and hybridization experiments test whether a behavior is inherited

In a deprivation experiment, an animal is reared so that it is deprived of all experience relevant to the behavior under study. In one such experiment, a tree squirrel was reared in isolation, on a liquid diet, and in a cage without soil or other particulate matter. When the young squirrel was given a nut, it put the nut in its mouth and ran around the cage. Eventually it made stereotypic digging movements in the corner of its cage, placed the nut in the imaginary hole, went through the motions of refilling the hole, and ended by tamping the nonexistent soil with its nose. The squirrel had never handled a food object and had never experienced soil, yet the stereotypic behavior of a squirrel burying a nut was fully expressed.

In a hybridization experiment, closely related species are interbred and the behavior of their offspring observed. Closely related species frequently show distinct differences in certain kinds of behavior. When such species can be interbred, it is possible to see whether their offspring have inherited elements of the behavior of one or both parents.

Konrad Lorenz, a pioneer in the field of ethology, conducted hybridization experiments on ducks to investigate the genetic determinants of their elaborate courtship displays. Dabbling duck species such as mallards, teals, pintails, and gadwalls are closely related to one another and can interbreed, but because of the specificity of their courtship displays, they rarely do so in nature. Each male duck performs a carefully choreographed water ballet that is typical of his species (Figure 52.2), and a female is not likely to accept his advances unless the entire display is successfully and correctly completed.

When Lorenz crossbred duck species, he found that the hybrid offspring expressed some components of the courtship displays of each parent species, but expressed them in new combinations. Of particular interest was his observation that the hybrids sometimes showed display components that were not in the repertoire of either parent species, but were characteristic of the displays of other species. Lorenz's hybridization studies clearly demonstrated that the motor patterns of the courtship displays were inherited. The fact that natural selection was shaping these genetically determined behaviors was evidenced by the fact that females were not interested in males performing hybrid displays.

52.2 Courtship Ballet of the Mallard

The courtship display of the male mallard duck contains about ten elements. The displays of closely related duck species contain some of the same ten elements, but have other elements not displayed by mallards. The elements of the courtship display and their sequence are species-specific and act to prevent hybridization.

A mounted immature male European robin with no red feathers does not stimulate aggression from a territorial adult male...



...but this formless clump of red feathers releases strong aggressive attacks from the territorial adult.



52.3 A Releaser of Aggressive Behavior

Red feathers serve as a releaser of aggressive behavior in male European robins.

Simple stimuli can trigger behaviors

If a behavior is not expressed during a deprivation experiment, it may nonetheless have genetic determinants. The right conditions may not have been available to stimulate the behavior during the experiment. The squirrel described above, for example, had to be given a nut for its digging and burying behaviors to be triggered. Specific stimuli are required to elicit the expression of many inherited behaviors. Two pioneering ethologists, Konrad Lorenz and Niko Tinbergen, who conducted classic studies of the nature of the stimuli that elicit such behaviors, called such stimuli releasers.

Releasers are usually a simple subset of all the sensory information available to an animal. Adult male European robins, for example, have red feathers on their breasts, which serve as releasers of aggressive behavior in other males. During the breeding season, the sight of an adult male robin stimulates another male robin to sing, perform aggressive displays, and attack the intruder if he does not heed these warnings. An immature male robin, whose feathers are all brown, does not elicit this aggressive behavior. A tuft of red feathers on a stick, however, is a sufficient releaser for male aggressive behavior in robins (Figure 52.3).

Tinbergen and A. C. Perdeck carefully examined the releasers involved in the interactions between herring gulls and their chicks during feeding. An adult herring gull has a

7. Head up, tail up

8. Turn toward female

^^

M^MAnZ

9. Nod swimming



10. Turn the back of the head

928 CHAPTER FIFTY-TWO

EXPERIMENT

Question: What characteristics of the herring gull parent release pecking responses from their chicks?

Adult herring gull

1. Paper cut-out models of gull heads with many variations were presented to chicks and their pecking responses were counted.

RESULTS

Stimulus:

Presence of red patch on bill Head shape



Head color

Bill alone

Bill shape

C

c-z- —

r g

u a

- c

I °

- _-



£>



£> ,&> . * .-'

Control:

LOO

62

35

91

125

98

99



94

64

A

vL

A red dot is more important than a realistic profile.

Head color and shape have little effect on the ability of a red dot to stimulate the pecking response.

jL

A red dot will elicit a response, even without a head.

Without a red dot, a long, thin bill elicits the strongest pecking response.

Conclusion: A contrasting dot on a thin bill releases pecking responses. Head shape and color have little or no influence.

52.4 Releasing the Pecking Response

A series of experiments rated the pecking responses of herring gull chicks to artificial models of gull heads to discover which features of the parent were releasers of this behavior.

red dot at the end of its bill (Figure 52.4). When the gull returns to its nest with food, the chicks peck at the red dot, thereby stimulating the adult to regurgitate the food for the chicks to eat.

Tinbergen and Perdeck hypothesized that the red dot was a releaser for the chicks' begging behavior. To test their hypothesis, they made paper cutout models of gull heads and bills, varying the colors and the shapes. Then they rated each model according to how many pecks it received from naive, newly hatched chicks (Figure 52.4). The shape or color of the model head made no difference. In fact, a head was not even necessary; the chicks responded just as well to models of bills alone—as long as they had the red dot. Surprisingly, the most effective releaser for chick pecking behavior was a long, thin

object with a dark tip that bore no resemblance to an adult herring gull. Clearly the chicks had inherited the ability to recognize a simple stimulus and respond to it with their also inherited begging behavior. To the ethologists, this represented an excellent example of a behavior that was genetically determined rather than learned.

Learning also shapes behavior

For their very significant contributions to our understanding of animal behavior, three ethologists, Lorenz, Tinbergen, and Karl von Frisch (whose work on honeybees you will encounter later in this chapter) shared a Nobel Prize in 1973. New generations of behavioral biologists, however, have moved beyond the ethologists' focus on inherited behavior to show that most behavior actually involves an interaction between inheritance and learning. The begging behavior of gull chicks is a case in point. Although newly hatched chicks respond maximally to simplistic artificial releasers, they gradually learn to discriminate between models and real gull heads, and they eventually beg only from their own parents. Thus, the inherited ability to recognize a simple releaser is subsequently refined by learning.

The early ethologists did not ignore learning or deny that it took place; in fact, they pioneered the study of learning. Tinbergen performed an early study of spatial learning, by which an animal learns to recognize features in its environment. In a classic experiment, he placed objects such as pine cones near the entrance of a nest dug by a female digger wasp. After the wasp left her nest, he moved the objects a short distance away. Upon returning, the wasp oriented to the moved objects and could not find her nest entrance (Figure 52.5). She had learned to recognize objects in the en-

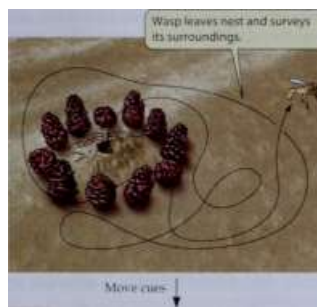
EXPERIMENT

Question: Does a wasp learn to locate its nest by visual cues?

METHOD Surround nest entrance with moveable visual cues, and move them to another location after the wasp leaves the nest and has surveyed its surroundings.

Wasp leaves nest and surveys surroundings.

II



RESULTS

j Wasp looks for nest entrance in relation to visual cues.



Conclusion: A wasp learns to use objects in its environment to locate its nest.

ANIMAL BEHAVIOR 929

52.5 Spatial Learning

Tinbergen's classic experiment showed that a female digger wasp learns the positions of objects in her environment.

interacting with him as if he were their parent (Figure 52.6). When the experiment was repeated by his assistants, each wearing boots with a different design, the goslings imprinted on the boots, and would follow only a person wearing the boots they first saw when they hatched.

The critical period for imprinting is determined by a developmental or hormonal state and can be quite brief. If a mother goat, for example, does not nuzzle and lick her newborn within 5 to 10 minutes after birth, she will not recognize it as her own later. In this case, imprinting depends on olfactory cues, and the critical period is determined by the high levels of the hormone oxytocin circulating in the mother at the time of birth.

Inheritance and learning interact to produce bird song

Many behavior patterns are intricate interactions of inheritance and learning. One example that has been the subject of some elegant experiments is bird song. Adult male songbirds use a species-specific song in territorial displays and courtship. A few species, such as the song sparrow, express their species-specific song even during deprivation experiments, but most species do not. For most species, such as the white-crowned sparrow, learning is an essential step in the acquisition of song.

If the eggs of white-crowned sparrows are hatched in an incubator and the young male birds are reared in isolation, their adult songs will be unusual assemblages of sounds, not the typical species-specific song. This species cannot express its species-specific song without being imprinted on

environment to use as orientation cues. More recently, spatial learning has been studied in animals such as squirrels, chickadees, and jays that cache food items in hundreds and even thousands of locations. Their capacity for learning and remembering where their food is cached is phenomenal.

Imprinting is the learning of a complex releaser

Releasers are generally simple subsets of the available information because there are limits to what can be programmed genetically. A type of learning called imprinting makes it possible to learn, during a limited critical period, a complex set of stimuli that can later serve as a releaser. The classic example is the imprinting of offspring on their parents and parents on their offspring to ensure individual recognition even in a crowded situation such as a colony or a herd.

When Lorenz incubated goose eggs in an incubator, and he was the first thing the goslings saw when they hatched, they imprinted on him, following him everywhere and in-



52.6 Imprinting Enables an Animal to Learn a Complex

Releaser

When Konrad Lorenz was the first thing newly hatched goslings saw, they imprinted on him, interacted with him as if he were their parent.

930 CHAPTER FIFTY-TWO

EXPERIMENT

Question: Is learning essential for song acquisition in white-crowned sparrows?

Control

METHOD

RESULTS

Raise young sparrows in the presence of an adult male sparrow singing. Record song of these control birds when they mature and plot as a sonogram.



36

= 14 r 15*3

Control or wild bird

<\\WMWv

0.5

Experiment 1

1.0 Time (seconds)

1.5

2.0

METHOD

RESULTS

Hatch eggs in an incubator and rear the birds in isolation. Record and plot their song. Compare to the control birds' song.

§ 6

$\wedge r g r 4$

a a. 3

$B_{jj} 2 * \wedge 1$

Isolated hand-reared bird

0.5

1.0

Time (seconds)

1.5

2.0

Conclusion: White-crowned sparrows that do not hear adult song as nestlings do not express the correct song when they mature. Therefore nestling birds learn a song template.

EXPERIMENT

Question: Do maturing white-crowned sparrows require auditory feedback to learn to express the correct song?

Experiment 2

METHOD

RESULTS

Deafen a subadult bird that has heard
the song of his father when he was a nestling.

9 6

6 I 4

p <u z

- g 1

Deaf bird

$v^{\wedge} W f h$



kkh



0.5 1.0 1.5

Time (seconds)

2.0

Conclusion: Even if the bird has the correct song template, he needs auditory feedback to learn to match it.

52.7 Two Critical Periods for Song Learning

To sing his species-specific song as an adult, a male white-crowned sparrow must acquire a song memory by hearing the song as a nestling, and must be able to hear himself as he attempts to match his singing to that memory.

that song as a nestling (Figure 52.7). But even though the male white-crowned sparrow must hear the song of his own species as a nestling to sing it as an adult, he does not sing it as a juvenile. Instead, he uses his auditory imprinting as a nestling to form a song memory in his nervous system. As the young male sparrow approaches sexual maturity the following spring, he tries to sing, and eventually he matches his imprinted song memory through trial and error. If a bird that has heard his species-specific song as a juvenile is deafened before he begins to express his song, he will not develop his species-specific song (see Figure 52.7). The bird must be able to hear himself to match his song memory. If he is deafened after he expresses his correct song, he will continue to sing like a normal bird. Two periods of learning are essential: the first in the nestling stage, the second as the bird approaches sexual maturity.

Genetically determined behavior is adaptive under certain conditions

The ability to learn and to modify behavior as a result of experience is often highly adaptive. Most human behavior is the result of learning. Why, then, are so many behavior patterns in so many species genetically determined? We have already touched on one answer to this question: If role models and opportunities to learn are not available— as in species with nonoverlapping generations, such as spiders—then there is no alternative to inherited behavior.

Inherited behaviors are also adaptive when mistakes are costly or dangerous. Mating with a member of the wrong species is a costly mistake; thus the function of much courtship behavior, such as that of dabbling ducks, is to guarantee species recognition. In an environment in which incorrect as well as correct models exist, learning the wrong pattern of courtship behavior would be possible.

Behavior patterns used to avoid predators or capture of dangerous prey allow no room for mistakes. If the behavior is not performed promptly and accurately the first time, there may not be a second chance (Figure 52.8).

Thus, inherited behavior is highly adaptive for species that have little opportunity to learn, for species that might learn the wrong behavior, and in situations in which mistakes are costly or dangerous.



52.8 Some Things Can't Be Learned by Trial and Error

In total darkness, the sound of a striking rattlesnake triggers an automatic escape jump in a kangaroo rat. The rat does not have to learn this behavior.

Hormones and Behavior

All behavior depends on the nervous system for initiation, coordination, and execution. Frequently, however, it is the endocrine system, through its controlling influences on the development and the physiological state of the animal, that determines when a particular behavior is performed, and even when certain behaviors can be learned. In this section we will present two complex cases in which hormones control the development, learning, and expression of behavior: sexual behavior in rats and maturation of the brain regions required for song learning and expression in birds.

Sex steroids determine the development and expression of sexual behavior in rats

Differences in the behavior of males and females of a species are clear examples of genes influencing the development and expression of behavior. Such sex differences in behavior are the result of actions of the sex steroids on the brain.

Rats, like most other animals, have stereotypic sexual behaviors. A female rat in estrus (receptive to males) responds

to a tactile stimulus of her hindquarters by assuming a mating posture called lordosis. A male rat encountering a female in estrus engages in stereotypic copulatory behavior. The roles of genes and sex steroids in the development and expression of lordosis and male copulatory behavior have been investigated through experiments that manipulated the exposure of the developing and adult rat brain to sex steroids.

Experiments such as those shown in Figure 52.9 led to three conclusions:

► Sex steroids are necessary for adult rats to express sexual behavior. Moreover, the male sex steroid, testosterone, has an effect only in males, and the female sex steroid, estradiol, has an effect only in females.

52.9 Hormonal Control of Sexual Behavior

In newborn rats of both sexes whose reproductive organs (ovaries or testes) have been removed, the presence of testosterone establishes male behavior patterns (no lordosis), and its absence establishes female patterns (lordosis).

EXPERIMENT

Question: Does exposure to testosterone soon after birth influence the development of sexual behavior in rats?

Experiment 1 (a) Experiments on female rats

Experiment 2 (b) Experiments on male rats

Spay a newborn ♀

Spay a newborn ♀ and treat her with testosterone



Castrate a newborn ♂

Castrate a

newborn ♂ and J// treat him with testosterone



Let her mature

Let her mature

Let him mature

Let him mature



\

I



Castrate an adult ♂

I

I



Treat with ♂ sex steroids

I

Observe: Lordosis

Treat with ♀ Treat with ♀

sex steroids sex steroids

\ I

Observe: Lordosis Observe: No lordosis

Treat with \$ sex steroids

Treat with 9 sex steroids

\

\



Observe: No lordosis tiff

Observe: Lordosis

Treat with 9 sex steroids

I

Observe: No lordosis



Treat with testosterone

Treat with testosterone

Treat with testosterone

Treat with testosterone

Treat with testosterone

Treat with testosterone

I

I

I

I

I

I

Observe: No sexual behavior

Observe: No sexual behavior

Observe: cT sexual behavior



Observe: Copulation Observe: No sexual behavior in presence of receptive 9

Observe: Copulation



Conclusion: In rats, the presence of testosterone establishes male behavior patterns, and its absence establishes female patterns.

932 CHAPTER FIFTY-TWO

► Development of male sexual behavior requires the brain of the new born rat to be exposed to testosterone, but development of female sexual behavior does not require the neonatal brain to be exposed to estradiol.

► Neonatal exposure to testosterone masculinizes the nervous systems of both genetic males and females so that they express male sexual behavior as adults.

Thus, the sex steroids that are present during development determine which pattern of sexual behavior develops, and the sex steroids that are present in adulthood determine whether that pattern is expressed.

Testosterone affects the development of the brain regions responsible for song in birds

As we saw above, learning is essential for the acquisition of bird song. Both male and female birds hear their species-specific song as nestlings, but only the males of most songbird species sing as adults. Male birds use song to claim territory, compete with other males, and declare dominance. They also use song to attract females, which suggests that the females know the song of their species even if they do not sing. Do sex steroids control the learning and expression of song in male and female songbirds?

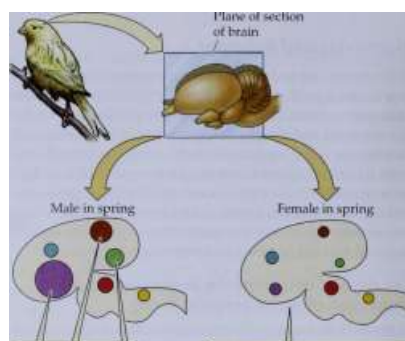
After leaving the nest where they heard their father's song, young songbirds from temperate and arctic habitats migrate and associate with other species in mixed flocks. During this time they do not sing, and they do not hear their species-specific song again until the following spring. As that spring approaches and the days become longer, the young male's testes begin to grow and mature. As his testosterone level rises, he begins to try to sing. Even if he is isolated at this time from all other males of his species, his song will gradually improve until it is a proper rendition of his species-specific song. At that point the song is crystallized—the bird expresses it in similar form every spring thereafter. The young male's brain has learned the pattern of the song by hearing his father. During the subsequent spring, under the influence of testosterone, he learns to express that song—a behavior that then becomes rigidly fixed in his nervous system.

Why don't the females of most songbird species sing? Can't they learn the patterns of their species-specific song? Do they lack the muscular or nervous system capabilities necessary to sing? Or do they simply lack the hormonal stimulus for developing the behavior? To answer these questions, investigators injected female songbirds with testosterone in the spring. In response to these injections, the females developed their species-specific song and sang just as the males did. Apparently females learn the song pattern of their species when they are nestlings and have the capability to express it, but they normally lack the hormonal stimulation.

What does testosterone do to the brain of the songbird? A remarkable discovery revealed that testosterone causes the parts of the brain necessary for learning and expressing song to grow larger (Figure 52.10). Each spring, certain regions of the males' brains grow. Individual neurons in-

Male canary

Plane of section of brain



Testosterone induces growth in the regions responsible for song.

During the nonbreeding season, the male bird's song regions are similar in size to those of a female's brain.

52.10 Effects of Testosterone on Bird Brains

In spring, rising testosterone levels in the male cause the song regions of the brain to develop. The size of each circle is proportional to the volume of the brain occupied by that region.

crease in size and grow longer extensions, and the numbers of neurons in those regions of the brain increase. Such research on the neurobiology of bird song has revealed that hormones can control behavior by influencing brain structure as well as brain function, both developmentally and seasonally.

The Genetics of Behavior

To say that behavior is inherited does not mean that specific genes code for specific behaviors. Genes code for proteins, and there are many complex steps between the expression of a gene as a protein product and the expression of a behavior. In no case are all the steps between a gene and its influence on a behavior known. Nevertheless, it is clear that behavior has genetic determinants. In this section we will look at three approaches to investigating how genes affect behavior: hybridization, artificial selection and crossing of the selected strains, and molecular analysis of genes and gene products.

Hybridization experiments show whether a behavior is genetically determined

The effects of hybridization on the courtship displays of duck species were the subject of a classic ethological experiment, as we saw above. A more recent set of hybridization experiments was performed on the songs of crickets. Crickets songs, like bird songs, are species-specific, and as in birds, only male crickets "sing." They do so by rubbing one wing against another that has a serrated edge. These sounds can be recorded and analyzed quantitatively.

When two species of crickets were crossed, their offspring (the F₁ generation) expressed songs that had features of the songs of the two parental species. Backcrosses of F₁ individuals with the parental species produced individuals that had songs closer to the parental species used in the back-cross. Clearly the genetic background determined the song pattern. What was amazing, however, was the demonstration that female preferences for male songs were under similar genetic control. Given a choice, females from each parental species preferred the calls of males from their own species, but hybrid females preferred the calls of hybrid males.

These genetic differences between the cricket species and the hybrids were reflected in the properties of their nervous systems. When specific neurons in the crickets' brains were stimulated, songs were expressed that reflected the genotypes of the crickets.

Artificial selection and crossbreeding experiments reveal the genetic complexity of behaviors

Domesticated animals provide abundant evidence that artificial selection of mating pairs on the basis of their behavior can result in strains with distinct behavioral as well as anatomical characteristics. Among dogs, consider retrievers, pointers, and shepherds. Each has a particular behavioral tendency that can be honed to a fine degree by training. However, dogs and other large animals are not the best subjects for genetic studies. Most artificial selection experiments in behavioral genetics have been done on more convenient laboratory animals with short life cycles and large numbers of offspring.

A favorite subject for behavioral genetic studies has been the fruit fly (*Drosophila*). Artificial selection has been successful in shaping a variety of behavior patterns in fruit flies, especially aspects of their courtship and mating behavior. Crossing of these artificially selected strains reveals that most of these behavioral differences are due to multiple genes that probably influence the behavior indirectly by altering general properties of the nervous system. Some single-gene effects, however, can be isolated. One example is the gene *per* (short for "period"), which alters the frequency of the wing vibrations that are part of the male's courtship display. The *per* gene is not a courtship behavior gene, however. It has subsequently been found that this gene codes for a transcription factor that plays an important role in the generation of daily rhythms of rest and activity, as we will see below. How it alters the development of wingbeat frequency is not clear.

Few behavioral genetic studies reveal simple Mendelian segregation of behavioral traits. An exception is nest-cleaning behavior in honeybees. One genetic strain of honeybees practices nest-cleaning, or hygienic, be-

havior, which makes them resistant to a bacterium that infects and kills the larvae of honeybees. When a larva dies, workers uncap its brood cell and remove the carcass from the hive. Another strain of honeybees does not show this hygienic behavior and therefore is more susceptible to the spread of the disease (Figure 52.11).

When these two strains of honeybees were crossed, the results indicated that the hygienic behavior was controlled by two recessive genes. All members of the F₁ generation were nonhygienic, indicating that the behavior is controlled by recessive genes. Backcrossing the F₁ with the hygienic strain produced the typical 3:1 ratio expected for a two-gene trait (see Chapter 10). The behavior of the nonhygienic hybrid individuals was very interesting. One-third of them showed no hygienic behavior at all; one-third uncapped the cells of dead larvae but did not remove them; and one-third did not uncapped cells, but did remove carcasses if the cells were open.

52.11 Genes and Hygienic Behavior in Honeybees

Some honeybee strains remove the carcasses of dead larvae from their nests. This behavior seems to have two components: uncapping the larval cell (u) and removing the carcass (r), each of which is under the control of a recessive gene.

Nonhygienic bees

Hygienic bees

Parental generation

Genotype of females Genotype of males Gametes (male or female)

F₁ (all nonhygienic)

Genotype of females Gametes produced by females

Backcross to males of hygienic strain

F₂ generation females



Hygienic bees uncap cells and remove the dead larvae.

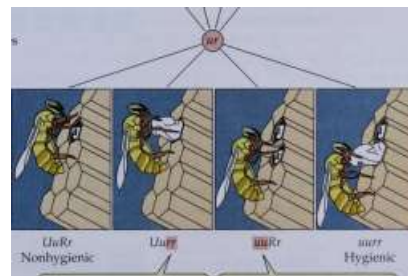
mm <: uu is the "uncap" gene. UY 1 rr is the "remove" gene.

(2) '

UuRr

@@@©

Genotypes Behaviors



These bees are nonhygienic, but will remove dead larvae if cells are uncapped.

These bees are nonhygienic. They will uncap cells of dead larvae, but won't remove them.

934 CHAPTER FIFTY-TWO

I \ en though these results appear to indicate a gene for uncapping and a gene for remo\ al, these behavior patterns are complex. [hey involve sensor) mechanisms, orientation mo\ ements, and motor patterns, each of which depends on multiple properties o\ many cells. The genetic deficits of nonhygienic bees could influence very small, specific, yet critical properties o\ some cells. If a single critical property, such as a crucial synapse or a particular sensory receptor, \ \ ere lacking, the whole behavior would not be expressed. The responsible gene, then, is not a specific gene that codes for the entire behavior.

Molecular genetics techniques reveal specific genes that influence behavior

Molecular geneticists are investigating specific genes that influence behaviors. Male courtship behavior in fruit flies {Drosophila) is a subject of many such studies. This behavior is stereotypic, species-specific, and requires no learning. Males recognize potential mates, follow them, tap the female's body with their forelegs, extend and vibrate one wing, and lick the female's genitals. If the female is receptive, the male copulates with her (Figure 52.12a). Research in molecular genetics has now shown that most of this male courtship behavior is controlled by a single gene.

In fruit flies with two X chromosomes (females), a gene

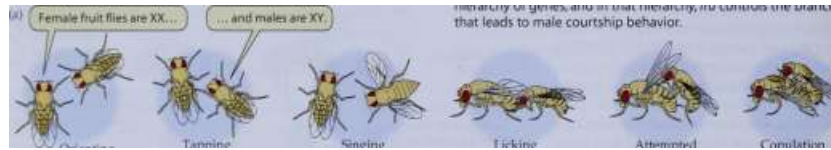
called sex-lethal (sxl) is expressed. This gene is at the top of a genetic hierarchy that determines all aspects of sexual differentiation and behavior (Figure 52.12fr). The Sxl protein causes another gene called transformer (tra) to produce the female-specific Tra protein. Fruit flies without the tra gene develop into males anatomically and behaviorally, regardless of how many X chromosomes they have. But it is still another gene in the sex determination hierarchy that is responsible for male behavior.

The Tra protein controls two additional genes called dou-blesex (dsx) and fruitless (fru). The dsx gene mostly controls the

anatomical differentiation of males, and fru causes the formation of a nervous system that expresses male courtship behavior. Mutations of the fru gene do not affect male body form, but they disrupt male courtship behavior. We don't know all of the actions that the male-specific Fru protein has in the development of the fruit fly nervous system, but this is about as close as we can get at present to identifying a gene that controls a complex behavior.

52.12 The fruitless Gene Controls Male Courtship Behavior in Fruit Flies

(a) Male fruit flies display stereotypic, species-specific courtship behavior. (fc>) Sexual differentiation in *Drosophila* is controlled by a hierarchy of genes, and in that hierarchy, fru controls the branch that leads to male courtship behavior.



(b)

f

Orienting

Tapping

Singing

Licking

Sex-determining pre-mRNAs are spliced in one specific way in female flies...

Female-specific mRNA

f

Attempted copulation

Copulation

...and another way in males.

Male-specific mRNA

§] Female *sxl* and *tra* mRNAs make proteins that control splicing in the expression of genes in the female-specific hierarchy.

Transcription and *sxl* mRNA splicing I I I I I T T T 1 -4-

X Female *Sxl* protein

sex-lethal (*sxl*)

Transcription and mRNA splicing

► on



o

"Stop codon transformer (*tra*)

Default splicing

t

K \

Q

TTT I II

No functional ^^ Q Male *sxl* and *tra* *Sxl* protein ^^ mRNAs have

stop codons that

Female *Tra* protein

}

Stop codon doublesex (dsx)

\

No functional Tra protein

terminate translation.

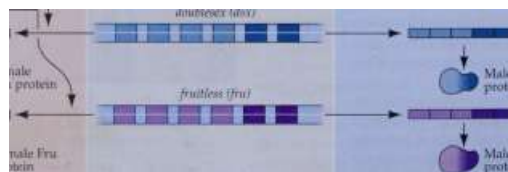
I I I

◆

O Female Dsx protein

I I 1 I

O Female Fru protein



Male Dsx protein

Q The default splicing of dsx mRNAs controls

A male anatomy...

Q ...and of fru, male courtship behavior.

Male Yxw⁺ protein

Communication

Communication is behavior that influences the actions of other individuals. It consists of displays or signals that can be perceived by other individuals and which convey information to them. Natural selection shapes displays or signals into systems of communication if the transmission of information benefits both the sender and the receiver. Thus, the ultimate cause of communication is the selective advantage it gives to individuals that engage in it. The courtship displays of a male, for example, benefit the male if they attract females, and they benefit the female if they allow her to assess whether the male is of the right species and whether he is strong, vigorous, and has other attributes that will make him a good father. A common mutual benefit of communication is the reduction of uncertainty about the status or intentions of the signaler. Even in aggressive interactions, reducing uncertainty helps both sender and receiver to avoid physical harm.

Studies of communication can be complex because they must take into account the sender, the receiver, and the environment. The displays or signals that an animal can generate depend on its physiology and anatomy. Likewise, an animal's ability to perceive displays or signals depends on its sensory physiology and on the environment through which the display or signal must be transmitted.

In Chapter 45, we learned how sensory systems function in chemosensation, tactile sensation, audition, vision, and electrosensation. These are the channels of animal communication. In the discussion that follows, we will explore each of these five channels in turn.

Chemical signals are durable but inflexible

Molecules used for chemical communication between individual animals are called pheromones. Because of the diversity of their molecular structures, pheromones can communicate very specific messages that contain a great deal of information. The mate attraction pheromone of the female silkworm moth is a good example (see Figure 45.4). Male moths as far as several kilometers downwind are informed by these molecules that a female of their species is sexually receptive. By orienting to the wind direction and following the concentration gradient of the molecules, they can find her.

Territory marking is another example in which detailed information is conveyed by chemical communication (Figure 52.13). Pheromonal messages left by mammals such as cats and dogs, for example, can reveal a great deal of information about the animal: species, individual identity, reproductive status, size (indicated by the height of the message), and when the animal was last in the area (indicated by the strength of the scent).

An important feature of pheromones is that once they are released, they remain in the environment for a long time. By contrast, vocal or visual displays disappear as soon as the animal stops signaling or displaying. The durability of pheromonal signals enables them to be used to mark trails, as ants do, or to indicate directionality, as in the



Panthera tigris

52.13 Many Animals Communicate with Pheromones

To mark her territory, this female tiger is spraying pheromonal secretions from a scent gland in her hindquarters onto a tree. Other tigers passing the spot will know that the area is "claimed," and they will know something about the animal who claimed it.

case of the moth sex attractant. However, it also means that the message cannot be changed rapidly. This inflexibility makes pheromonal communication unsuitable for a rapid exchange of information.

The chemical nature and the size of the pheromonal molecule determine its speed of diffusion. The greater the speed of diffusion, the more rapidly the message gets out and the farther it will reach, but the sooner it will disappear. Trail-marking and territory-marking pheromones tend to be relatively large molecules that diffuse slowly; sex attractants tend to be small molecules that diffuse rapidly.

Visual signals are rapid and versatile but are limited by directionality

Visual signals are easy to produce, come in an endless variety, can be changed very rapidly, and clearly indicate the position of the signaler. However, the extreme directionality of visual signals means that they are not the best means of getting the attention of a receiver. The receptors of the receiver must be focused on the signaler, or the message will be missed. Most animals are sensitive to light and can therefore receive visual signals, but sharpness of vision limits the detail that can be transmitted. The complexity of the environment also limits visual communication.

Because visual communication requires light, it is not useful at night or in environments that lack light, such as caves and the ocean depths. Some species have surmounted this constraint on visual communication by evolving their own light-emitting mechanisms. Fireflies use a enzymatic mechanism to create flashes of light. By emitting flashes in species-specific patterns, fireflies can advertise for mates at night.

936 CHAPTER FIFTY-TWO

Fireflies also illustrate how some species can exploit the communication systems of other species. There are predator - of fireflies that mimic the mating flashes of

other species. When an eager suitor approaches the signaling individual, it is eaten. Thus, deception can be part of animal communication systems, just as it is part of human

Auditory signals communicate well over a distance

Compared with visual communication, auditory communication has advantages and disadvantages. Sound can be used at night and in dark environments. It can go around obstacles that would interfere with visual signals, so it can be used in complex environments like forests. It is better than visual signals at getting the attention of a receiver because the receiver does not have to be focused on the signaler for the message to be received. Like visual signals, sound can provide directional information, as long as the receiver has at least two receptors spaced somewhat apart. By maximizing or minimizing the features of the sounds they emit, animals can make their location easier or more difficult to determine.

Sound is useful for communicating over long distances. Even though the intensity of sound decreases with distance from the source, loud sounds can be used to communicate over distances much greater than those possible with visual signals. An extreme example is the communication of whales. Some whales, such as the humpback, have very complex songs. When these sounds are produced at a certain depth (around 1,000 m), they can be heard hundreds of kilometers away. In this way, humpback whales can locate each other over vast areas of ocean.

Auditory signals cannot convey complex information as rapidly as visual signals can, as the expression "A picture is worth a thousand words" implies. When individuals are in visual contact, an enormous amount of information is exchanged instantaneously (for example, species, sex, individual identity, reproductive status, level of motivation, dominance, vigor, alliances with other individuals, and so on). Coding that amount of information, with all of its subtleties, as auditory signals would take considerable time, thus increasing the possibility that the communicators could be located by predators.

The animal world is relatively silent. Most invertebrates do not produce sound; cicadas and crickets are marvelous exceptions. Many amphibians, most fishes, and most reptiles produce no sound.

Tactile signals can communicate complex messages

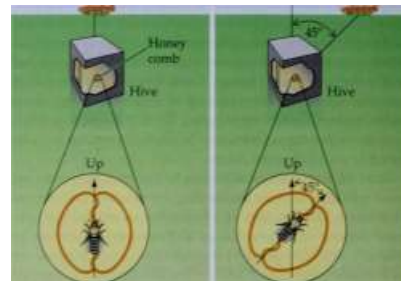
Communication by touch is extremely common, although not always obvious. Animals in close contact use tactile interactions extensively, especially under conditions that do not favor visual communication. When eusocial insects such as ants, termites, or bees meet, they contact one another with their antennae and front legs. One of the best-studied uses of tactile communication, beginning with the pioneering work of ethologist Karl von Frisch, is the dance of honeybee. When a forager bee finds food, she returns to

(a)

(b)

Food source

Food source



Pattern of waggle dance

Partem of waggle dance

ffl



52.74 The Waggle Dance of the Honeybee (a) By running straight up on the surface of the honeycomb in a dark hive, a honeybee tells her hivemates that there is a food source in the direction of the sun and at least 80 meters from the hive. The intensity of the waggle indicates exactly how far the food source is. If the food source were in the opposite direction from the sun, she would orient her waggle runs straight down, (b) When her waggle runs at an angle from the vertical, the other bees know that the same angle separates the direction of the food source from the direction of the sun.

the hive and communicates her discovery to her hivemates by dancing in the dark on the vertical surface of the honeycomb. The dance is monitored by other bees, who follow and touch the dancer to interpret the message.

If the food is less than 80-100 meters from the hive, the forager performs a round dance, running rapidly in a circle and reversing her direction after each circumference. The odor on her body indicates the flower to be looked for, but the dance contains no information about the direction to go—only that it is within 100 meters of the hive.

If the food source is farther than 80-100 meters, the bee performs a waggle dance, which conveys information about both the distance and the direction of the food source. The bee repeatedly traces out a figure-eight pattern as she runs on the vertical surface. She alternates half-circles to the left and right with vigorous wagging of her abdomen in the short, straight run between turns. The angle of the straight run indicates the direction of the food source relative to the direction of the sun (Figure 52.14). The speed of the dancing

ANIMAL BEHAVIOP 937

indicates the distance to the food source: The farther away it is, the slower the waggle run.

Electric signals can also communicate messages

Some species of fish have evolved the ability to generate electric fields in the water around them by emitting a series of electric pulses (see Chapter 45). These trains of electric pulses can be used for sensing objects in the immediate surroundings, and they can also be used for communication.

An electrode connected to an amplifier and a speaker can be used to "listen" to the signals generated by glass knife fish in a tank. Each individual fish emits a pulse at a different frequency, and the frequency each fish uses relates to its status in the population. Males emit lower frequencies than females. The most dominant male has the lowest frequency, and the most dominant female has the highest frequency. When a new individual is introduced into the tank, the other individuals adjust their frequencies so that they do not overlap, and the signal of the new individual indicates its position in the hierarchy. In their natural environment—the murky waters of tropical rainforests—these fish can tell the identity, sex, and social position of another fish by its electric signals.

Communication has been a very fruitful area for investigating the ultimate causes of behavior and how the resulting adaptations have been shaped by the environment. Next we will return to some studies of proximate causes of behavior to see some examples of how "how" questions can be addressed.

The Timing of Behavior: Biological Rhythms

Among the important proximate causes of behavior are those that determine its organization through time. The study of biological rhythms has led to major discoveries about brain mechanisms down to the molecular level that enable animals to organize their behavior in time. In the discussion that follows, we will examine two types of biological rhythms: circadian rhythms and circannual rhythms.

Circadian rhythms control the daily cycle of behavior

Our planet turns on its axis once every 24 hours, creating a cycle of environmental conditions that has existed throughout the history of life. Daily cycles are characteristic of almost all organisms. What is surprising, however, is that this daily rhythmicity does not depend on the 24-hour cycle of light and dark.

If animals are kept in constant darkness, at a constant temperature with food and water available all the time, they still demonstrate daily cycles of activity sleeping, eating, drinking, and just about anything else that can be measured. This persistence of the daily cycle in the absence of changes between light and dark suggests that animals have an endogenous (internal) clock. Without time cues from the environment, however, these daily cycles are not exactly 24 hours long. They are therefore called circadian

rhythms from the Latin *circa*, "about," and *diēs*, "day".

To discuss biological rhythms, we must introduce some terminology. A rhythm can be thought of as a series of cycles, and the length of one of those is the period of

the rhythm. Any point on the cycle is a phase of that cycle:

Rhythm

Period



Time

Hence, when two rhythms completely match, they are in phase, and if a rhythm is shifted (as in the resetting of the clock), it is phase-advanced or phase-delayed. Since the period of a circadian rhythm is not exactly 24 hours, it must be phase-advanced or phase-delayed even day to day to remain in

phase with the environment.

entrainment. The process of resetting of the circadian

rhythm by environmental cues is called entrainment. An animal kept in constant conditions will not be entrained to the 24-hour cycle of the environment, and its circadian clock will run according to its natural period—it will be free-running. If its period is less than 24 hours, the animal will begin its activity a little earlier each day (see the middle panel of Figure 52.15).

Animals with free-running circadian rhythms can be used in experiments to investigate the stimuli that phase-shift or entrain the circadian clock. Under natural conditions, environmental cues, such as the onset of light or dark, entrain the free-running rhythm to the 24-hour cycle of the real world. In the laboratory, it is possible to entrain circadian rhythms in free-running animals with short pulses of light or dark administered every 24 hours (bottom panel of Figure 52.15).

When you fly across several time zones, your circadian clock is out of phase with the real world at your destination; the result is jet lag. Gradually your endogenous rhythm synchronizes itself with the real world as it is entrained by environmental cues. Since your endogenous

rhythm cannot be shifted by more than 30 to 60 minutes each day, it takes several days to reentrain your clock to real time in your new location. This period of reentrainment is the time during which you experience jet lag, because your endogenous rhythm is waking you up, making you sleepy, initiating activities in your digestive tract, and stimulating many other physiological functions at inappropriate times of the day.

the circadian clock. Where is the clock that controls the circadian rhythm? In mammals, the master circadian clock is located in two tiny groups of cells just above the optic chiasm, the place where the two optic nerves cross. These structures are called the supra chiasmatic nuclei (SCN).

938 CHAPTER FIFTY-TWO

EXPERIMENT

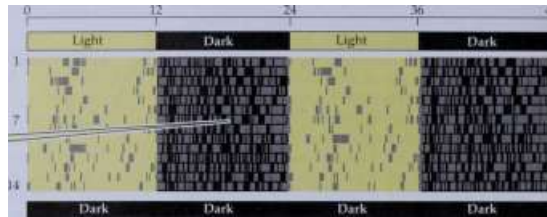
Question: Do daily patterns of rest and activity depend on a 24-hour light-dark cycle?

METHOD Vary the light-dark cycle and record the activity of a mouse on an exercise wheel.

RESULTS

Days 1 l

On a cycle of 12 h light/12 h dark, the mouse is mostly active in the dark and has a rest-activity cycle of 24 hours.



In constant dark, the mouse still expresses a daily cycle of rest and activity, but the period of the cycle is less than 24 hours.

21

28

lllllllll lla Mini l lll! Hill ■ 1111111 l

ii in ii tin ii mil inn

in hiuiii ■ mi i in nun in ■ ■ hi

un la i iiiii ii ii an uiiii in i ii

■ i iininii i nun in un i i

II HIIIBIIU mtt mrrnu ii I mi lllll 11o IIBIB I

i hi minium minimi

11un mi ii iiw ■ in i

! II II! II III linn I III _ I I I III II11 IIIII lll IIIII

If the mouse is given 20 minutes of light at 24-hour intervals, its rest-activity cycle is entrained to a 24-hour period.



illinium tun i win in iiwiii iiiii iiiii nun in in ■ ■ in an i m ii ■ mill mmum uni in i iiiii ii nan mil Dim

i iimna'lgiaiiiiia ibhi [iiiiiiiiini ma ibiiii a linn inn ■ muni i II II llll II nun IBHII

i linn in in iii mii

i ■ iiiii i i ii inn II in ■ laim iiiii in iiiiiiiii i in iiiiiiiiii ii iaiiii i

ii ■ mini inn wi i ■ ■ a i hi iiiii ■ iii niiiia

a i in ii i ii ii a i ii

i ii in mil ii i n in i iia

iaiiiiitiiiiiaiii mnia i i ii

I ii in un i ■ ii i iiiii i hiuiii ii i ii iiniif

IIIIII I

In in i

Conclusion: The mouse has an endogenous rest-activity cycle of less than 24 hours, but that cycle can be entrained by the 24 h daily cycle of light and dark.

52.75 Orcadian Rhythms

The marks indicate times when a mouse is running on an activity wheel. Two days of activity are recorded on each horizontal line, such that the data for each day are plotted twice, once on the right half of a line and again on the left half of the next line below; this double plotting makes patterns easier to see. The schedule of light and dark exposure is indicated by the solid bars running across the figure. First the mouse experiences 12 hours of light and 12 hours of dark every day (top panel), then it is placed in constant darkness (middle panel), and finally it is given a 20-minute exposure to light each day (bottom panel). In constant darkness, the circadian rhythm is free-running, but a 20-minute flash of light at 24-hour intervals can entrain it.

the SCN are destroyed, the animal loses circadian rhythmicity. Under constant conditions, the animal is equally likely to be active or asleep at any time of day (Figure 52.16).

Recent experiments have shown that circadian rhythms

of rest and activity can be restored in an animal whose SCN have been destroyed. It receives a transplant of those nuclei

from another animal. In no other known case can a brain transplant restore such a complex behavior. Since the restored rhythm has the period of the animal that donates the tissue, the transplant clearly controls the recipient's behavior.

Circadian rhythms are found in every animal group, as well as in protists, plants, and fungi, but only vertebrates have SCN. Thus, natural selection has produced a variety of circadian clocks. In the mollusk *Bulla*, for example, the cells driving circadian behavior are in the eyes. Birds do have SCN, but the master clock of at least some species resides in the pineal gland, a mass of tissue between the cerebral hemispheres that produces the hormone melatonin. If the pineal gland of a bird is removed, the bird loses its circadian rhythm. In protists and fungi, circadian rhythmicity is a property of individual cells, and the individual cells of many multicellular animals can generate circadian rhythms. What are the molecular mechanisms of these circadian clocks?

EXPERIMENT

Question: Is the suprachiasmatic nucleus (SCN) the site of the circadian clocks in mammals?

METHOD

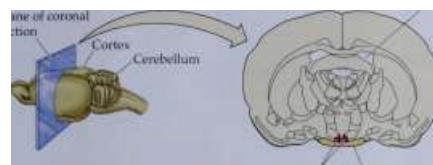
RESULTS

(1) Record the activity of a rat in constant darkness.

(2) Destroy its SCN and observe its activity patterns.

Coronal section

Plane of coronal section



Ventricle

SCN

Optic chiasm

The circadian clock of this animal runs naturally on a period greater than 24 hours.

Days 1

Destroying the SCN eliminates the circadian rhythm.



Conclusion: If the SCN is destroyed, a mammal loses its circadian rhythm

52.76 Where the Clock Is

The circadian clock of mammals is in the suprachiasmatic nuclei (SCN) of the brain. If its suprachiasmatic nuclei are destroyed, a mammal loses its circadian rhythm.

clock genes. Enormous progress has been made in recent years toward discovering the molecular basis of circadian rhythms. The surprise is that there is a high degree of homology in the genes involved across a very wide range of organisms, from bread molds to humans. The story begins with a gene called *period* that was discovered in fruit flies, as mentioned above. Mutations of this gene cause flies to have either short or long circadian periods. Mutations of another circadian gene, called *timeless* (short for "timeless"), cause a loss of circadian rhythms in fruit flies. The presence of mRNA for *period* and *timeless* shows a daily cycle, as does the presence of the *Per* and *Tim* proteins. Thus, the transcription and translation of these two genes shows a circadian rhythm. But what controls the rhythm? The *Per* and *Tim* proteins dimerize in the cytoplasm, and the resulting

heterodimer is translocated into the nucleus, where it acts as a transcription factor inhibiting the transcription of the *per* and *tim* genes (Figure 52.17). These two genes could thus be the wheels of a

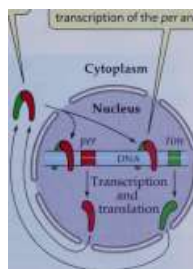
It is now known that the mechanism is not so simple, and that there are a number of other genes involved. What is interesting, however, is that homologies have been found between these clock genes in fungi, insects, mice, and humans, indicating how fundamental molecular clock mechanisms are to living organisms on Earth.

Transcription and translation of *per* and *tim* show circadian rhythms.

Per and *Tim* proteins dimerize in the cytoplasm.

U
o
12
24
36

The dimer is translocated to the nucleus where it inhibits further transcription of the *per* and *tim* genes.



Hours

52.17 Circadian Rhythms May Be Generated by a Molecular Clock

The *per* and *tim* genes discovered in fruit flies are homologous to "clock genes" found in a wide range of organisms. These genes are transcribed and translated on a circadian rhythm that seems to be controlled by positive feedback.

940 CHAPTER FIFTY-TWO

Circannual rhythms control seasonal behaviors

In addition to turning on its axis every 24 hours, our planet revolves around the sun once every 365 days. Because Earth is tilted on its axis, its revolution around the sun results in seasonal changes in day length at all locations except the equator. These changes secondarily create seasonal changes in temperature, rainfall, and other variables. Because the behavior of animals must adapt to these seasonal changes, animals must be able to anticipate the seasons and adjust their behavior accordingly. Most animals, for example, should not produce young in the winter.

For many species, change in day length, or photoperiod, is a reliable indicator of seasonal changes to come. If day length has a direct effect on the physiology and behavior of a species, that species is said to be photoperiodic. If male deer, for example, are held in captivity and subjected to two cycles of change in day length in one year, they will grow and drop their antlers twice during that year.

For some animals, change in day length is not a reliable cue. Hibernators spend long months in dark burrows underground, away from any indicators of day length, but have to be physiologically prepared to breed almost as soon as they emerge in the spring. A bird overwintering in the tropics cannot use changes in photoperiod as a cue to time its migration north to the breeding grounds. Hibernators and equatorial migrants have endogenous annual rhythms, called circannual rhythms. In other words, their nervous systems have a built-in calendar. Just as circadian rhythms are not exactly 24 hours long, circannual rhythms are not exactly 365 days long, but usually shorter. The brain mechanisms of circannual rhythms are completely unknown.

Finding Their Way: Orientation and Navigation

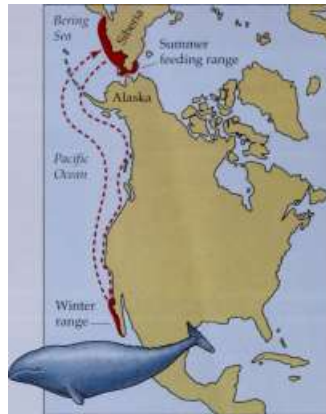
Within a local environment, finding your way is not a problem. Like the wasps Tinbergen studied, you remember landmarks and organize your behavior spatially with respect to those reference points. Such orientation is a very common animal behavior. But what if the destination is a considerable distance away? How does an animal orient to it and find its way?

Many animals navigate long distances through unfamiliar territory. In this section we describe modes of navigation and examine some of their underlying mechanisms.

Piloting animals orient themselves by means of landmarks

In most cases an animal finds its way using simple means: It knows and remembers the structure of its environment. It uses

landmarks to find its nest, a safe hiding place, or a food source. Navigating by means of landmarks is called piloting. Gray whales, for example, migrate seasonally between the Bering Sea and the coastal lagoons of Mexico. They find their way by following the west coast of North America (Figure 52.18). Coastlines, mountain chains, rivers, water currents, and wind patterns can all serve as piloting



"fc

o.Q

Summer ^*

feeding range



52.78 Piloting

Gray whales migrate south in winter from the Bering Sea to the coast of Baja California by following the coast of North America.

cues. But some remarkable cases of long-distance orientation and movement cannot be explained by piloting.

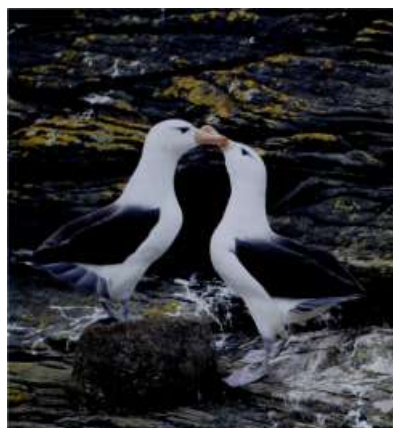
Homing animals can return repeatedly to a specific location

The ability of an animal to return to a nest site, burrow, or other specific location is called homing. In most cases, homing is merely piloting in a known environment, but some animals are capable of much more sophisticated homing.

People who breed and race homing pigeons take the pigeons from their home loft and release them at a remote site where they have never been before. The first pigeon that reaches its home wins the race. Data on departure directions, known flying speeds, and distances traveled show that homing pigeons fly fairly directly from the point of release to home. They do not randomly search until they encounter familiar territory.

Scientists have used homing pigeons to investigate the mechanisms of navigation. One series of experiments tested the hypothesis that the pigeons depend on visual cues. Pigeons were fitted with frosted contact lenses so that they could see no details other than degree of light and dark. These pigeons still homed and fluttered down to the ground in the vicinity of their loft. Thus, they were able to navigate without visual images of the landscape.

Marine birds provide many dramatic examples of homing over great distances in an environment where landmarks are rare. Many marine birds fly over hundreds of miles of featureless ocean on their daily feeding trips and then return directly to a nest site on a tiny island. Alba-



Dioinalcn melanophris

52.19 Coming Home

A pair of black-browed albatrosses engage in courtship display over their partially completed mud nest. Many albatrosses return to the site of their own birth to find a mate, and will return to that site year after year.

trosses display remarkable feats of homing. When a young albatross first leaves its nest on an oceanic island, it flies widely over the southern oceans for 8 or 9 years before it reaches reproductive maturity. At that time, it flies back to the island where it was raised to select a mate and build a nest (Figure 52.19). After the first mating season, the pair separate, and each bird resumes its solitary wanderings. The next year they return to the same nest site at the same time, reestablish their pair bond, and breed. Thereafter they return to the nest to breed every other year, spending many months in between at sea.

Migrating animals travel great distances with remarkable accuracy

For as long as humans have inhabited temperate and subpolar latitudes, they must have been aware that whole populations of animals, especially birds, disappear and reappear seasonally—that is, they migrate. Not until the early nineteenth century, however, were patterns of migration established by marking individual birds with identification bands around their legs. Being able to identify individual birds in a population made it possible to demonstrate that the same birds and their offspring returned to the same breeding grounds year after year, and that these same birds were found during the nonbreeding season at locations hundreds or even thousands of kilometers from the breeding grounds.

How do migrants find their way over such great distances? A reasonable hypothesis is that young birds on their

ANIMAL BEHAVIOR 941

first migration follow experienced birds and learn the landmarks by which they will pilot in subsequent years. However, adult birds of many species leave the breeding grounds before the young have finished fattening and are ready to begin their first migration. These naive birds must be able to navigate accurately on their own, and with little room for mistakes.

Navigation is based on internal and environmental cues

Since many homing and migrating species are able to take direct routes to their destinations through areas they have never experienced, they must have mechanisms of navigation other than piloting. Humans use two systems of navigation: distance-and-direction navigation and bicoordinate navigation. Distance-and-direction navigation requires knowing the direction to the destination and how far away that destination is. With a compass to determine direction and a means of measuring distance, humans can navigate. Bicoordinate navigation, also known as true navigation, requires knowing the latitude and longitude (the map coordinates) of both the current position and the destination. From that information, a route can be plotted to the destination.

distance-and-direction navigation. Researchers conducted an experiment with European starlings to determine their method of navigation. These birds migrate between their breeding grounds in the Netherlands and northern Germany and their wintering grounds to the southwest, in southern England and western France (Figure 52.20). The researchers captured birds on their breeding grounds, marked them, transported them to Switzerland—south of their breeding grounds—and released them. The researchers expected that if the starlings were using distance-and-direction navigation, the marked birds would be recovered in France and Spain, to the southwest of where they were released. Naive juvenile starlings did use distance-and-direction navigation, but experienced adult birds were less disrupted by their geographic displacement.

How do animals determine distance and direction? In many instances, determining distance is not a problem as long as the animal recognizes its destination. Homing animals recognize landmarks and can pilot once they reach familiar areas. Evidence suggests that biological rhythms play a role in determining migration distances for some species. Birds kept in captivity display increased and oriented activity at the time of year when they would normally migrate. Such migratory restlessness has a definite duration, which corresponds to the usual duration of migration for the species. Since distance is determined by how long an animal moves in a given direction, the duration of migratory restlessness could set the distance for its migration.

Two obvious means of determining direction are the sun and the stars. During the day, the sun is an excellent compass, as long as the time of day is known. In the Northern Hemisphere, the sun rises in the east, sets in the west, and

942 CHAPTER FIFTY-TWO

EXPERIMENT

Question: Do European starlings migrate from brooding to winter ranges using distance-and-direction navigation or bicoordinate navigation?

METHOD

RESULTS

Capture young birds before their first winter migration.

Mark birds and move them to a distant location and release them.

Record where they are recovered.



52.20 Distance-and-Direction Navigation

European starlings normally make a short winter migration in a southwesterly direction, from the Netherlands to coastal France and southern England (red arrow). Experimental populations of starlings moved to a site in Switzerland did not fly northwest to their traditional wintering grounds, but followed the same southwesterly route (blue arrow), which took them to Spain.

Starlings from Switzerland did not fly northwest to their traditional grounds, but followed the same southwesterly route, which took them to Spain.

Cf^— 7 U]

Conclusion: Juvenile starlings use distance-and-direction navigation.

points south at noon. As we have seen, animals can tell the time of day by means of their circadian clocks. Clock-shifting experiments have demonstrated that animals use their circadian clocks to determine direction from the position of the sun.

Researchers placed birds in a circular cage that enabled them to see the sun and sky, but no other visual cues (Figure 52.21). Food bins were arranged around the sides of the cage, and the birds were trained to expect food in the bin at one particular direction—south, for example. After training, no matter what time they were fed, and even if the cage was rotated between feedings, the birds always went to the bin at the southern end of the cage for food, even if that bin contained no food.

Next, the birds were placed in a room with a controlled light cycle, and their circadian rhythms were phase-shifted by turning the lights on at midnight and off at noon. After about 2 weeks, the birds' circadian clocks had been phase-advanced by 6 hours. Then the birds were returned to the circular cage under natural light conditions, with sunrise at 6:00 a.m. Because of the shift in their circadian rhythms, their endogenous clocks were indicating noon at the time the sun came up.

If food was always in the south bin, and it was sunup, the birds should have looked for food 90 degrees to the right of the direction of the sun. But since their circadian clocks were telling them it was noon, they looked for food in the direction of the sun—in the east bin. The 6-hour phase shift in their circadian clocks resulted in a 90-degree error in their orientation. These kinds of experiments on many species have shown that animals can orient by means of a time-compensated solar compass.

Many animals are normally active at night; in addition, many day-active bird species migrate at night and thus cannot use the sun to determine direction. The stars offer two sources of information about direction: moving constellations and a fixed point. The positions of constellations change because Earth is rotating. With a star map and a clock, direction can be determined from any constellation. But one point that does not change position during the night is the point directly over the axis on which Earth turns. In the Northern Hemisphere, a star called Polaris, or the North Star, lies in that position and always indicates north.

Stephen Emlen at Cornell University investigated whether birds use these sources of directional information from the stars. He raised young birds in a planetarium, where star patterns are projected on the ceiling of a large, domed room. The star patterns in the planetarium could be slowly rotated to simulate the rotation of Earth. If the star patterns were not rotated, birds caught in the wild could orient well in the planetarium, but birds raised in the planetarium under a nonmoving sky could not. If the star patterns in the planetarium were rotated each night as the young birds matured, they were able to orient in the planetarium, showing that birds can learn to use star patterns for orientation if the sky rotates (Figure 52.22).

These experiments provided no evidence that the birds used their circadian clocks to derive directional information from the star patterns. Experienced birds were not confused by a still sky, or by a sky that rotated faster than normal. These birds were orienting to the fixed point in the sky, the North Star. Young birds raised under a sky that rotated around a different star imprinted on that star and oriented to it as if it were the North Star. These studies showed that

K

EXPERIMENT

Question: How can pigeons determine compass direction from the sun, whose position changes with the time of day?

Experiment 1

METHOD

A pigeon placed in a circular cage from which it can see the sky (but not the horizon) can be trained to seek food in one direction, even when its cage is rotated between trials.

Sun

Food bins



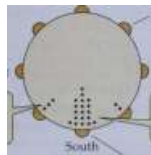
North

RESULTS

West

Each dot

represents a peck in search of food.



Food bin

East

A bird is trained to seek food in the south.

South

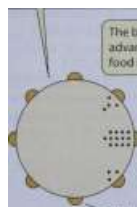
Full of food

Experiment 2

METHOD

A bird is placed on altered light-dark cycle and its circadian rhythm phase-advanced by 6 hours. The bird is then returned to the training cage under natural sky.

RESULTS



The bird with 6-hour phase-advanced rhythm now seeks food in the east.

East

Full of food

Conclusion: Pigeons have the ability to tell directions by means of a time-compensated solar compass.



Birds whose circadian rhythms were phase-shifted forward by 6 hours oriented as though the dawn sun was at its noon position. These results showed that birds are capable of using their circadian clocks to determine direction from the position of the sun.

birds raised in the Northern Hemisphere learn a star map that they can use for orientation at night by imprinting on the fixed point in the sky.

Animals cannot use sun and star compasses when the sky is overcast, yet they still home and migrate under such conditions. Do other sources provide information they can use for orientation? There appears to be considerable redundancy in animals' abilities to sense direction. Pigeons are able to home as well on overcast days as on clear days, but this ability is severely impaired if small magnets are attached to their heads—evidence that the birds use a magnetic sense. Cells have been found in birds that contain small particles of the magnetic mineral magnetite, but the neurophysiology of the magnetic sense is largely unknown. Another possible cue is the plane of polarization of light, which can give directional information even under heavy cloud cover. Very low frequencies of sound can provide information about coastlines and mountain chains. Weather patterns can also provide considerable directional information.

BiCOORDiNATE navigation. Bicoordinate navigation involves knowing where you are (in longitude and latitude) and where you want to go, and plotting an appropriate course. Longitude can be determined by the position of the sun and the time of day: If the sun comes up earlier than expected, you must be east of where you want to be, and if the sun comes up later than expected, then you are west of



52.22 Star Patterns Can Be Altered in a Planetarium

This scientist has placed birds in a planetarium. By changing the positions or movements of the stars projected on the planetarium ceiling, he can investigate what information the birds use for orientation.

944 CHAPTER FIFTY-TWO

where you want to be. Time and sun position can give information about latitude as well. At a given time of day in the Northern Hemisphere, a sun position higher in the sky than expected indicates you are farther south than you want to be, and if the sun is lower in the sky than expected, you are north of where you want to be. Information about longitude and latitude can also come from sensing Earth's magnetic lines of force and from the positions of the stars.

In spite of the remarkable navigational abilities of animals such as albatrosses, there is currently no evidence that animals use bicoordinate navigation. But, of course, it is not easy to do experiments on animals such as albatrosses!

Human Behavior

As we saw early in this chapter, the behavior of an animal is a mixture of components that are inherited and components that can be molded by learning. However, even some aspects of learned behavior patterns—such as what can be learned and when it can be learned—have genetic determinants. Thus natural selection shapes not only the physiology and morphology of a species, but also its behavior. In some situations natural selection favors inherited behaviors; in others, learned behaviors. In many cases, the optimal adaptation is a mixture of inherited and learned behavioral components. Given these considerations, how would we characterize human behavior?

An important characteristic of human behavior is the extent to which it can be modified by experience. The transmission of learned behavior from generation to generation—culture—is the hallmark of humans. Nevertheless, the structures and many functions of our brains are inherited, including drives, limits to and propensities for learning, and even some motor patterns. Biological drives such as hunger, thirst, sexual desire, and sleepiness are inherent in our nervous systems. Is it reasonable, therefore, to expect that emotions such as anger, aggression, fear, love, hate, and jealousy are solely the consequences of learning?

Our sensory systems enable us to use certain subsets of information from the environment; similarly, the structure of our nervous systems makes it more or less possible to process certain types of information. Consider, for example, how basic and simple it is for an infant to learn spoken language, yet how many years that same child must struggle to master reading and writing. Verbal communication is deeply rooted in our evolutionary past, whereas reading and writing are relatively recent products of human culture.

Some motor patterns seem to be programmed into our nervous systems. Studies of diverse human cultures from around the world reveal basic similarities of facial expressions and body language among human populations that have had little or no contact with one another. Infants born blind still smile, frown, and show other facial expressions at appropriate times, even though they have never observed such expressions in others.

Acknowledging that aspects of our behavior have been shaped through evolution in no way detracts from the

value we place on our ability to learn and the importance of cultural transmission of information to our species. Even so, we are recognizing that culture, in its simplest form, is not uniquely human. In the introduction to this chapter we saw what has been characterized as pre-cultural behavior in Japanese macaques. Individuals invented new behaviors, and those new behaviors were transmitted by imitative learning through the population.

In a recent study, scientists who have spent years studying chimpanzee behavior in seven widely separated areas of Africa compared their findings on chimpanzee behavior. They were able to identify 39 behaviors, ranging from tool use to courtship behavior, that were common in some populations but absent in others. Moreover, the variation in these behaviors was much greater between populations than within a population, and each population had a distinct repertoire of these behaviors. Just as human societies are characterized by different assemblages of culturally transmitted customs or customary behaviors, so are these chimpanzee populations.

It is increasingly more difficult to draw a line between human behavior and animal behavior, especially that of our closest primate relatives. But why should we expect such a line to exist? We do not expect such a lack of continuity in molecular, biochemical, physiological, or anatomical characteristics. Similarly, human and animal behavior is on a continuum, and the challenge is to understand the common mechanisms and the reasons for quantitative differences.

Chapter Summary

What, How, and Why Questions

- Studies of behavior seek to describe behaviors, understand their mechanisms, and understand their evolution.

Behavior Shaped by Inheritance

- Many behaviors of many species are stereotypic and species-specific, and are thus largely determined by inheritance. They do not require learning and are minimally modifiable by learning.
- Deprivation experiments deprive an animal of opportunities to learn a behavior and can therefore reveal that a behavior is hereditary.
- Hybridization experiments can also reveal genetic influences on behavior. Review Figure 52.2
- Some behaviors are triggered by simple stimuli called releasers. Review Figure 52.3, 52.4
- Spatial learning enables an animal to learn and use information about its physical environment. Review Figure 52.5
- Imprinting enables an animal to learn the features of a complex releaser, such as the identity of its parents. Review Figure 52.6
- The acquisition of bird song is an example in which genetic determinants and learning interact, enabling an animal to learn a behavior by focusing on the correct stimuli at the correct times. Review Figure 52.7
- Genetically programmed behavior is highly adaptive for species, such as those with nonoverlapping generations, that have little opportunity to learn, for species that might learn

ANIMAL BEHAVIOR 945

the wrong behavior, and in situations in which mistakes are costly or dangerous.

Hormones and Behavior

- In rats, the sex steroids present during development determine what sexual behavior patterns develop, and the sex steroids present in the adult control the expression of those patterns. Review Figure 52.9
- In birds, testosterone determines a bird's ability to sing by causing the brain regions responsible for song to develop. Review Figure 52.10

The Genetics of Behavior

- There are many complex steps between the expression of a gene as a protein product and the expression of a behavior. Several types of experiments help reveal how genes affect behavior.
- Artificial selection and crossbreeding can produce individuals with particular behavioral traits that are inherited. Review Figure 52.11
- The techniques of molecular genetics can reveal the functions of specific genes that influence behavior. Review Figure 52.12

Communication

- Communication consists of displays or signals, that can be perceived by other individuals and which influence their behavior. Natural selection favors communication systems when both sender and receiver benefit from the exchange of

information.

- ▶ The evolution of communication signals is constrained by the anatomical and physiological characteristics of a species that are available to be shaped by natural selection.
- ▶ Many animals communicate by emitting pheromones into the environment and by sensing the pheromones of other animals. Pheromonal messages can last a long time, but they cannot be changed quickly.
- ▶ Visual communication is easy, versatile, and rapid, but it is limited by its directionality, by the visual acuity of the receiver, and by environmental conditions such as darkness. Many animals communicate via visual signals.
- ▶ Auditory signals can be used at night, can go around objects that would interfere with visual communication, can easily get the receiver's attention, can provide directional information, and can travel long distances. Compared with visual communication, however, auditory communication is slow. Few animals communicate with auditory signals.
- ▶ Tactile signals can communicate complex messages, as the dance of the honeybee demonstrates. Review Figure 52.14
- ▶ The electric signals generated by some fishes can be used for communication.

The Timing of Behavior: Biological Rhythms

- ▶ Animal behaviors are expressed in daily cycles called circadian rhythms. A circadian rhythm is an endogenous rhythm with a period not equal to 24 hours. To remain in phase with the 24-hour daily cycle of the environment, a circadian rhythm must be phase-shifted every day. Phase-shifting cues such as the onset of light and dark entrain circadian rhythms to the natural 24-hour period. Review Figure 52.15
- ▶ In mammals, the clock that controls the circadian rhythm is located in the suprachiasmatic nuclei of the brain. In other animals, different structures function as the circadian clock. Review Figure 52.16
- ▶ Two genes have been identified that are involved in the clock mechanism in a variety of species. Review Figure 52.17
- ▶ Circannual rhythms ensure that animals, such as hibernators and equatorial migrants, that cannot rely on changes in day length as seasonal cues perform the appropriate behaviors at the appropriate times of year.

Finding Their Way: Orientation and Navigation

- ▶ Piloting animals find their way by orienting to landmarks. Review Figure 52.18
- ▶ Homing animals find their way through unfamiliar territory to specific locations. Migrating animals travel long distances with remarkable accuracy.
- ▶ Animals that navigate by distance and direction determine distance in part by recognizing landmarks in the vicinity of their destination and in part by biological rhythms timing how far they travel.
- ▶ Sources of directional information include a time-compensated solar compass and an ability to locate the fixed point in the nocturnal sky. Review Figures 52.20, 52.21
- ▶ The long-distance movements of some species are difficult to explain by distance-and-direction navigation mechanisms. Information for bicoordinate navigation is available from the physical environment, but there is no evidence that any species uses such information.

Human Behavior

- ▶ Like that of all other animals, human behavior consists of genetically determined and learned components. What sets humans apart from other animals is the extent to which we can modify our behavior on the basis of experience.

For Discussion

1. An oystercatcher is a bird that normally lays a clutch of two eggs. If you place an artificial nest with either three artificial but normal-sized eggs or one very large artificial egg near the oystercatcher's nest, the oystercatcher will abandon its own two eggs and attempt to incubate the artificial eggs. How can you explain this behavior?
2. Cowbirds are nest parasites. A female cowbird lays her eggs in the nest of another species, which then incubates the eggs and raises the young. What do you think would characterize the acquisition of song in cowbirds? In a given area, cowbirds tend to parasitize the nests of particular bird species. How do you think female cowbirds learn this behavior? How would you test your hypothesis?
3. The short-tailed shearwater is a bird that winters in Antarctica and summers in the Arctic. What problems would this species have in using either the sun or the stars for navigation? What is the most likely means it uses to find its way to its summer and its winter feeding grounds?
4. Male dogs lift a hind leg when they urinate; female dogs squat. If a male puppy receives an injection of estrogen when it is a newborn, it will never lift its leg to urinate for the rest of its life; it will always squat. How might this result be explained?
5. If you were able to be the first person to visit a human population that had never been in contact with another culture, how

could you use that opportunity to explore whether there were any human behaviors that were genetically determined?

Part Seven

Ecology and

BIOGEOGRAPHY



53

Behavioral Ecology

_*-

For a predator such as a cheetah, it starts to run away but then it usually slows down a bit and performs

a behavior called stotting. It jumps about half a meter off the ground with all four legs held straight and its white rump patch fully everted. Why would an animal that is fleeing from a predator slow down rather than speed up?

Several hypotheses have been proposed to explain why stotting may have evolved. First, the gazelle may be warning other nearby gazelles—particularly its relatives—that it has spotted a predator. Second, stotting individuals in a fleeing herd might distract and confuse a predator. Third, a stotting gazelle may be signaling to the predator that it has been seen and therefore pursuit will not be profitable.

Predictions based on these hypotheses have been tested in the field. The first and second hypotheses can be rejected because even solitary gazelles usually stot when they spot a predator. Only the third hypothesis is supported by existing data: Cheetahs, the most important predators on gazelles, are significantly more likely to abandon a pursuit if a gazelle stots than if it does not.

Individuals of all species, not only gazelles, interact in various ways with individuals of their own and other species and with their physical environments. The task of ecology is to understand the nature and consequences of these interactions. Ecologists may study these interactions by formulating and testing hypotheses, as they did for stotting in gazelles. Ecologists also study patterns of distribution and abundance of organisms to determine how these patterns are established and maintained, and how they change over short and long time periods. From its roots in descriptive natural history, ecology has developed into a complex field of inquiry dealing with levels of organization ranging from relationships of individual organisms with their physical and biological environments to the structure of communities and ecosystems.

As used by ecologists, the term environment includes physical and chemical factors such as water, nutrients, light, temperature, and wind. It also includes biotic factors: all other organisms that influence the lives of individuals. Because species are adapted for life in many different environments, their interactions with their biotic and abiotic environments are also varied. An environmental factor that

A Stotting Gazelle

Although it is being pursued by a cheetah, this Thomson's gazelle (*Gazella thomsoni*) has slowed down to jump high off the ground.

exerts a strong influence on individuals of one species may have no influence on individuals of another species.

Interactions between organisms and their environments are two-way processes. Organisms both influence and are influenced by their environments. Indeed, managing environmental changes caused by our own species is one of the major

problems of the modern world. For this reason, ecologists are often asked to help analyze causes of environmental problems and to assist in finding solutions for them. However, it is important not to confuse the science of ecology with the term "ecology" as it is often used in popular writing, referring to nature as a kind of superorganism.

In this first chapter on ecology, we will discuss behavioral ecology: how animals make decisions* that influence their survival and reproductive success, how ecologists study these decisions, and what they have learned from their studies.

Animals decide where to carry out their activities and how to select the resources they need—food, water, shelter, nest sites. Animals also respond to predators and competitors, and decide how to interact with other members of their own species (called conspecifics). Individual choices are the foundation of much of ecology, because changes in densities and distributions of populations are the cumulative results of the decisions of myriad individuals.

The use of the words "decision" and "decide" here does not imply that the choices animals make are conscious, but rather that these choices influence their survival and reproductive success and thus are molded by natural selection.



948 CHAPTER FIFTY-THREE

Balancing Costs and Benefits of Behaviors

In their attempts to understand the evolution of behavior, ecologists often analyze their observations in terms of costs and benefits. A cost-benefit analysis is based on the principle that an animal has only a limited amount of time and energy to devote to its activities. Animals generally do not perform behaviors whose total costs are greater than the sum of their benefits—the improvements in survival and reproductive success that the animal achieves by performing the behavior.

Of course, animals do not consciously calculate costs and benefits, but over many generations, natural selection molds behavior in accordance with costs and benefits. A cost-benefit approach provides a framework that behavioral ecologists can use to make observations and design experiments that enable them to understand why behavior patterns evolve as they do.

The cost of behaving has three components. The energetic cost of a behavior is the difference between the energy the animal would have expended had it rested and the energy expended in performing the behavior. The risk cost of a behavior is the increased chance of being injured or killed as a result of performing it compared with resting. The opportunity cost of a behavior is the sum of the benefits the animal forfeits by not being able to perform other behaviors during the same time interval. Opportunity costs measure the fitness trade-offs involved in performing different behaviors for different amounts of time. An animal that devotes all of its time to foraging, for example, does not achieve high reproductive success!

During the mating season in September and October, male Yarrow's spiny lizards maintain territories from which they exclude conspecific males. Behavioral ecologists assessed the costs of this behavior by stimulating territorial behavior in the lizards during June and July, a time of year when the lizards normally are only weakly territorial. They did this by inserting small capsules containing testosterone beneath the skin of some males. Control males were also captured and released, but received no testosterone implants.

The testosterone-implanted males patrolled their territories more, performed more threat displays, and expended about one-third more energy (energetic cost) than control

males. As a result, they had less time to feed (opportunity

cost), captured fewer insects, stored less energy, and died at a higher rate (risk cost) (Figure 53.1).

This experiment demonstrated that the costs of active territorial defense in June and July probably explain why the lizards normally reduce territorial behavior at that time of year. Presumably, during the mating season, when females are receptive and territorial behavior can result in increased reproductive success, its benefits are great enough to offset these costs.

Choosing Where to Live and Forage

Selecting a place in which to live is one of the most important decisions an individual makes. Where an individual chooses to live and how it uses that environment strongly

EXPERIMENT

Question: Why are male Yarrow's spiny lizards only weakly territorial in the summer?

METHOD Insert testosterone capsules under skin of

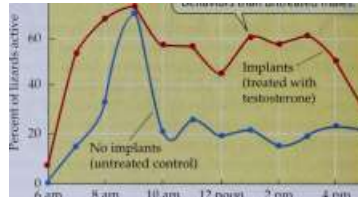
males during the summer and observe their behavior and survivorship.

Yarrow's spiny lizard



y 60

Testosterone-treated males were more active and displayed more territorial behaviors than untreated males.



10 am 12 noon 2 pm Time of day

4 pm

100

Treated males survived less well than untreated males.

S 60

01

40

No implants (untreated control)



Implants (treated with testosterone)

10 20 30 40

Time after receiving implant (days)

50

Conclusion: If lizards are territorial in the summer, they die at a higher rate than nonterritorial lizards.

53.1 The Costs of Defending a Territory

By using testosterone implants to increase territorial behavior, experimenters measured the costs to male Yarrow's spiny lizards of defending a territory during the summer months, when females are not receptive.

influence its survival and reproductive success. In this section, we will consider how animals choose environments that provide adequate food and shelter, and how they select their food.

Features of an environment may indicate its suitability

The environment in which an organism normally lives is called its habitat. Once a habitat is chosen, an animal seeks its food, resting places, nest sites, and escape routes from predators within that habitat.

The cues organisms use to select suitable habitats are as varied as the organisms themselves, but all habitat selection cues have a common feature: They are good predictors of general conditions suitable for future survival and reproduction. A simple example of habitat selection cues is that of the red abalone, a gastropod mollusk that begins its life

f

as a fertilized egg in the open ocean. The egg hatches about 14 hours after fertilization, and a motile larva emerges with enough yolk to continue developing for another 7 days. During this time it swims in the open water without eating. At the end of 7 days, the larva stops developing, swims to the seafloor, chooses a place in which to settle, and metamorphoses into an adult. ¹ Abalone larvae settle only on coralline algae, upon which they feed. They recognize coralline algae by the presence of a specific chemical—a water-soluble peptide containing about 10 amino acids—that the algae produce. In the laboratory, abalone larvae will settle on any surface on which this chemical has been placed, but in nature only coralline algae produce it. By using this simple cue, these larvae always settle on a surface that is suitable for their future development.

Red abalone grow to adulthood feeding on a single patch of coralline algae; their habitat is their food. However, most animals must make many choices about where and how to seek and select food after they have settled in a habitat.

How do animals choose what to eat?

Because food is such an important resource, we consider it here in some detail. When an animal is looking for food, how much time should it spend searching in one area before moving to another site? When many different types of prey are available, which ones should a predator take, and which ones should it ignore? Foraging theory attempts to provide answers to these questions.

To construct a hypothesis about how a foraging animal should behave, a scientist first specifies the objective of the behavior and then attempts to determine the behavioral choices that would best achieve that objective. This approach is known as optimality modeling. Its underlying assumption is that natural selection has molded the behavior of animals so that they solve problems by making the best choices available to them. Many hypotheses are possible, because a forager may be attempting to maximize the rate at which it obtains energy, to get enough vitamins or minerals, or to minimize its risk while foraging.

As an example, consider how a predator should choose among the available prey. Let's assume that the predator chooses prey so as to maximize its rate of energy intake. This is a reasonable objective because the more efficiently a predator captures food, the more time and energy it will have for other activities, such as reproduction. Therefore, a more efficient predator should produce more offspring than a less efficient one, and animals should evolve to make prey

choice decisions that maximize their rate of energy intake.

To test this hypothesis, we can characterize each type of prey available to the predator by two features: the amount of time it takes the predator to pursue, capture, and consume one of them, and the amount of energy an individual prey item contains. We then rank the prey according to the amount of energy the predator gets relative to the amount of time it spends pursuing, capturing, and handling the prey. The most valuable prey type is the one that yields the $\frac{\text{energy}}{\text{time}}$ most energy per unit of time invested.

With this information, we can build an optimality model to determine the rate at which a predator would obtain energy given a particular prey selection strategy. We can then compare alternative foraging strategies and determine the one that yields the highest rate of energy intake. Such calculations show that, if the most valuable prey type is abundant enough, a predator gains the most energy per unit of time spent foraging by taking only the most valuable prey type and ignoring all others. However, as the abundance of the most valuable prey type decreases, an energy-maximizing predator adds less valuable prey types to its diet in order of the energy per unit of time that those prey yield.

Ecologists performed laboratory experiments with bluegill sunfish to measure the energy content of different prey types (water fleas of different sizes), the time needed to capture and eat different prey types, the energy spent pursuing and capturing prey, and actual encounter rates with prey under different prey densities.

Using these measurements, the investigators predicted the proportions of large, medium, and small water fleas that bluegills would capture in environments stocked with different densities and proportions of those prey types (Figure 53.2). Based on the optimality model, they predicted

EXPERIMENT

Question: Do bluegills select prey to maximize their energy intake?

METHOD Provide bluegills with (1) varying proportions of

Daphnia (water fleas) of different sizes and (2) differing abundance (density) of food supply (Daphnia). Compare prey actually eaten with theoretical predictions.

Q Density of Daph



Daphnia

Bluegill

Conclusion: Bluegills select prey to maximize their rate of energy intake.

53.2 Bluegills are Energy Maximizers

In an experiment, the prey choices of bluegill sunfish were very similar to those predicted by an optimality model based on the goal of maximizing the rate of energy intake.



950 CHAPTER FIFTY-THREE

that in an environment stocked with low densities of all three types of prey, the fish would take every water flea that they encountered, but that in an environment with abundant large water fleas, the bluegills would ignore smaller water fleas.

To test their predictions, the investigators put the bluegills in environments containing three different prey densities and observed the proportions of the water fleas of different sizes they actually captured. The proportions of large, medium, and small water fleas taken by the fish were very close to those predicted by the model. Such tests of foraging theory using many different kinds of animals have provided ecologists with a set of rules showing how animals find and choose their prey. They have also provided estimates of the energetic costs and benefits of foraging behavior.

Mating Tactics and Roles

Individual animals choose their associates, how to interact with them, and when to leave them. The most important choice of associates and a male makes; is- male's preference

Mating behavior involves only a small set of choices. The most basic mating decision is choosing a partner of the correct species. Once that has been determined, additional decisions can be based on the qualities of a potential mate, on the resources it controls—food, nest sites, escape places—or on a combination of the two. Among those species in which individuals do not control any resources, the traits of the partner are the only criteria for mate selection. Here we will discuss how individuals choose their mating partners and show why males and females approach courtship so differently.

Abundant sperm and scarce eggs drive mating behavior

The reproductive behavior of males and females is often very different. Males usually initiate courtship, and they often fight for opportunities to mate with females. Females seldom fight over males, and they often reject courting males. Why do males and females approach courtship and copulation so differently?

The answer lies in the costs of producing sperm and eggs. Because sperm are small and cheap to produce, one male produces enough to sire a very large number of offspring—usually many more than the number of eggs a female can produce or the number of young she can nourish. Therefore, males of most species can increase their reproductive success by mating with many females.

Eggs, on the other hand, are typically much larger than sperm and are expensive to produce. Consequently, a female is unlikely to increase her reproductive output very much by increasing the number of males she mates with. The reproductive success of a female depends primarily upon the quality of the genes she receives from her mate, the resources he controls, and the amount of assistance he provides in the care of her offspring. Thus, females choose among males based on these criteria. By their

EXPERIMENT

Question: Did sexual selection affect the evolution of long tails in African long-tailed widowbirds?

METHOD Artificially lengthen or shorten tails on birds by cutting feathers or gluing on feathers.

RESULTS

rz

5

u

a.

&l

3 c

QJ

bC

CO 14

>

<

Males whose tails were artificially lengthened attracted more females and had greater reproductive success...



...than males with normal or shortened tails.



Artificially lengthened

Normal

Artificially shortened

Conclusion: Sexual selection in widowbirds favors long tails.

53.3 The Longer the Tail, the Better the Male

Male widowbirds with shortened tails defended their display sites successfully but attracted fewer females than males with long tails.

choices, females may cause the evolution of exaggerated traits that signal male quality. Why

Sexual selection often leads to exaggerated traits

Traits may evolve among individuals of one sex as a result of sexual selection: the selection of traits that confer advantages to their bearers during courtship or when they compete for mates or resources. Successful competitors for resources may gain exclusive access to mates that are attracted to the resources they control. Traits that improve success in courtship may evolve as a result of mating preferences by individuals of the opposite sex.

"Sexual selection is responsible for the evolution of the remarkable tails of African long-tailed widowbirds, which are longer than their heads and bodies combined. Male widow-birds compete for display sites, at which they perform courtship displays to attract females. To examine the role of the tail in sexual selection, an ecologist shortened the tails of some males by cutting them, and lengthened the tails of others by gluing on additional feathers. Both short-tailed and long-tailed males successfully defended their display sites, indicating that the long tail does not confer an advantage in male-male competition. However, males with artificially elongated tails attracted about four times more females than males with shortened tails (Figure 53.3).

Why do females prefer males with long tails? Probably because the ability to grow and maintain a long tail, which

(a)

This male song sparrow is in territory-defense posture.

Sparrow territories are approximately 50-100 m in diameter.

Each number identifies an individual male bird and its territory.



Field

Road



(b) *Morus bassanus*

53.4 Some Territories Provide Everything; Others Provide Only a Nest Site

(a) Male song sparrows defend large breeding territories that contain nesting sites, food resources, and protective cover, (b) The size of a breeding territory among these northern gannets is determined by how far an incubating bird can reach to peck its neighbors without leaving its eggs.

probably carries energetic costs, indicates that the male is vigorous and healthy. Why, then, don't male widowbirds have even longer tails than they do? A likely answer is that the costs of producing a longer tail would exceed the benefits, but the costs of long tails were not measured in these experiments.

Males attract mates in varied ways

Males employ a variety of tactics to induce females to copulate with them. Males of some species defend territories that contain food, nesting sites, or other resources. Some territories are all-purpose: They provide mating sites, nest-

ing sites, and the food necessary to rear offspring (Figure 53.4rt). Other territories include a breeding and nesting area, but do not supply all of the food necessary to rear young. The territories of many seabirds, such as gannets, penguins, and cormorants, are very, small, consisting of only the area that individuals can defend while sitting on their nests (Figure 53.4/7).

If a male controls no resources, he may use courtship behavior that signals in some way that he is in good health, that he is a good provider of parental care, or that he has a good genotype. For example, males of some species of hangingflies court females by offering them dead insects. By capturing an insect and defending it from other males, he demonstrates his ability as a forager and as a competitor. A female hangingfly will mate with a male only if he provides her with food. The bigger the food item, the longer she copulates with him, and the more of her eggs he fertilizes (Figure 53.5).

Whether a male fertilizes the eggs of a female with whom he has copulated depends on when they copulate and whether she copulates with other males. Males have evolved behavior patterns that increase the probability that it will be their sperm and no other male's that fertilize a female's eggs. The simplest method is to remain with the female for as long as she is fertile and prevent other males from copulating with her, but this method has high opportunity costs because a male can not do anything else while he is guarding a female.

Males of many species have evolved behaviors that are more elaborate but take less time. A male black-winged damselfly grabs a female and, using his penis, scrubs out any sperm other males have deposited in her sperm storage chamber. The male removes 90-100 percent of competing sperm before he inserts his own sperm into the chamber. Males of some other insects deposit a plug that effectively seals the opening to the female's genital chamber and prevents other sperm from entering.



53.5 A Male Wins His Mate

The male hangingfly has just presented a moth to his mate, thus demonstrating his foraging skills. She feeds on the moth while they copulate.

952 CHAPTER FIFTY-THREE



The throat feathers of a male bluethroat reflect ultraviolet light.

Females are the choosier sex

As we have seen, females can improve their reproductive success if they can assess the genetic quality and health of potential mates, the quality of the resources they control, and the quantity of parental care they may provide. But how can females make such assessments without the advantage of males to attempt to signal that they are good in all three of these traits?

By paying particular attention to those signals at which males cannot cheat, females have favored the evolution of "reliable" signals. Possession of a large dead insect indicates that a male hangingfly is a good forager and competent provider. Likewise, a male widowbird with a very long tail is likely to be a high-quality mate.

Like tail length, the brightness of the plumage of birds may indicate their health and genetic quality. Male blue-throats (small Eurasian thrushes) have bright blue throat patches (Figure 53.6). Investigators in Norway tested the hypothesis that females use the throat patch as a sign of male quality by blackening the throats of some males and then measuring those males' success in fertilizing eggs. Although most birds form pair bonds, they sometimes engage in extra-pair copulations, so that some of the eggs in a nest may be fertilized by a male other than the one who attends it. A higher proportion of eggs in the nests of males whose throats had been blackened were fertilized by "other" males than of eggs in the nests of control males.

The investigators also tested the role of the ultraviolet-reflecting "blue" males that had copulated with a female were more reflective of the blue feathers on mate choice by female compared to defend her nest against predators, than males that bluethroats. They reduced the UV reflectance of some males that had not copulated with her. Males also set females with males by applying to their blue patches a mixture of fat and UV-absorbing sunblock. Inexperienced copulated look for food on their territories.

Luscinia svecica

53.6 Ultraviolet Reflectance Affects Mate Choice

Female bluethroats are attracted to males whose throat feathers have high reflectance, which signals a healthy, high-quality mate.

In nests of red-winged blackbirds in Washington state were fathered by a male other than the owner of the territory in which the nest was located. All the other fathers were males holding nearby territories: fertile females went to those territories and solicited copulations from the males.

Females that copulated with more than one male, raised more offspring than females that remained faithful to their partner. Their reproductive success improved because neighbors

from the glands the birds use to oil their own feathers and UV-absorbing sunblock. Control males received the fat coating with no sunblock. Although the two groups of males looked the same to human observers, female bluethroats could distinguish them. Females started laying eggs sooner on the territories of control males, and they preferred control males as partners for extra-pair copulations. These experiments show that females can use subtle clues to assess the quality of males.

Social and genetic partners may differ

Behavioral ecologists have known for many years that animals nearly always copulate with their mates—the individuals

with which they have established pair bonds—but that they sometimes also copulate with other individuals. However, until the recent development of DNA fingerprinting methods, investigators had to assume that mated individuals were the parents of the offspring they raised.

By using the new molecular methods to compare the genomes of offspring with those of their supposed parents and other individuals, ecologists have found that nestling birds are nearly always the offspring of the female attending the nest—that is, females rarely lay eggs in other females' nests. However,

Also, there were fewer infertile eggs in nests with multiple fathers than in nests with single fathers. Females try to prevent their mates from copulating with other females, but they must leave them unguarded at times, both to feed and to seek extra-pair copulations of their own.

Costs and Benefits of Social Behavior

Social behavior evolves when individuals that cooperate with others of the same species have, on average, higher rates of survival and reproductive success than those achieved by solitary individuals. Associations for mating may consist of little more than a coming together of eggs and sperm, but individuals of many species associate for longer times to provide care for their offspring. Associating with conspecifics may also improve survival for reasons unrelated to reproduction, such as by reducing the risk of being captured by a predator.

We describe only a few animal social systems, but these examples demonstrate two important concepts. First, social systems are best understood not by asking how they

benefit the species as a whole, but by asking how the individuals that join together benefit. Second, social systems exist in a single nest often. Individuals constantly communicate with others. For example, 34 percent of nestlings one another and adjust their relationships.

a>



53.7 Individuals Hunting Together Can Subdue Large Prey

By hunting as a group, lionesses can kill larger animals than a single female could subdue alone.

Group living confers benefits and imposes costs

Living in groups may confer many types of benefits on individuals. It may improve hunting success or expand the range of prey that can be captured. For example, by hunting together, social carnivores improve their efficiency in bringing down prey (Figure 53.7). Such cooperative hunting was a key component of the evolution of human social behavior. By hunting in groups, our ancestors were able to kill large mammals they could not have subdued alone. These social humans could also defend their prey and themselves from other carnivores.

Many small birds form flocks. To find out whether flocking provides protection against predators, an investigator released a trained goshawk near wood pigeons in England. The hawk was most successful in capturing a pigeon when it attacked solitary pigeons. Its success decreased as the number of pigeons in the flock increased (Figure 53.8). The larger the flock of pigeons, the sooner some individual in the flock spotted the hawk.

Living in a group typically imposes costs as well as benefits. Individuals in groups may compete for food, interfere with one another's foraging, injure one another's offspring, inhibit one another's reproduction, or transmit diseases to their associates.

The effects of group living on the survival and reproductive success of an individual also depend on its age, sex, size, and physical condition. Individuals may be larger or smaller than the average for their age and sex. Variation in skills, competitive abilities, and attractiveness to potential mates often associated with these size differences.

An almost universal cost associated with group living is higher exposure to diseases and parasites. Long before the causes of disease were known, people knew that association with sick persons increased their chances of getting sick. Quarantine has been used to combat the spread of illness for as long as we have written records. The diseases of wild animals are not well known, but most of those that have been studied are spread by close contact.

EXPERIMENT

Question: Does flocking confer antipredator benefits?

METHOD

f



Wood pigeon

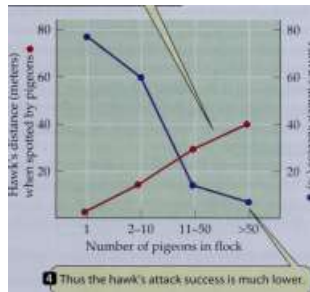
Release hawks near pigeon flocks of different sizes.



RESULTS

Q Observe whether a hawk I captures a pigeon.

I The more pigeons in the flock, the sooner the hawk is spotted and the pigeons initiate evasive action.



1 2-10 11-50 >50

Number of pigeons in flock

I Thus the hawk's attack success is much lower

Conclusion: Flocking provides protection against predation.

53.8 Flocking Provides Defense against Predators

The larger a flock of pigeons, the greater the distance at which they detect an approaching hawk, and the less likely the hawk is to succeed in capturing a pigeon.

Categories of Social Acts

Individuals living together perform many behaviors that are not performed by solitary animals. These acts can be grouped into four categories according to their effects on the interacting individuals:

- ▶ An altruistic act benefits another individual at a cost to the performer. — \ ■*—
- ▶ A selfish act benefits the performer but inflicts a cost on some other individual. —W—
- ▶ A cooperative act benefits both the performer and the recipient. a, v
- ▶ A spiteful act inflicts costs on both the performer and the recipient. _ —

954 CHAPTER FIFTY-THREE

Act benefits the performer (+)

Act costs the performer

(-)

53.9 Types of Social Acts

Social acts can be divided into four categories, based on their effects on the performer and the recipient.

The types of social acts are summarized in Figure 53.9. The terms used are purely descriptive; they do not imply conscious

motivation or awareness on the part of the animal. If a genetic basis for a cooperative or selfish behavior exists, and if performing it increases the fitness of the performer, then the genes governing that behavior will increase in frequency in the lineage. In other words, cooperative or selfish behavior can evolve.

How can behavior that inflicts a cost on the performer evolve? Behavioral ecologists believe spiteful behavior is rare in nature. Altruistic behavior, however, can evolve—both among close relatives and among unrelated individuals.

Altruism can evolve by means of natural selection

Altruistic behaviors evolve most easily when performers and recipients are genetically related. Genetic relatedness is important because an individual can influence its fitness in two different ways. First, it may produce its own offspring, contributing to its individual fitness. Second, it may help its

relatives in ways that increase their fitness.

—'

"Because relatives are descended from a common ancestor, they are likely to be carrying some of the same alleles. Two offspring of the same parents, for example, are likely to share 50 percent of the same alleles; an individual is likely to share 25 percent of its alleles with its sibling's offspring. Therefore, by helping its relatives, an individual can increase the representation of some of its own alleles in the population. This process is called kin selection. Together, individual fitness and kinship determine the inclusive fitness of an individual. Occasional altruistic acts may

eventually evolve into altruistic behavior patterns if the benefits of increasing the reproductive success of relatives

53.70 White-Fronted Bee-Eaters Are Altruists

Bee-eaters that help to care for nestlings preferentially help close relatives.

re

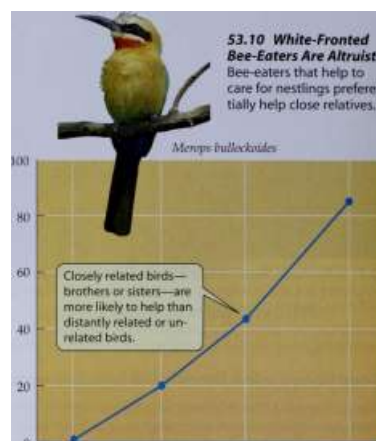
3

-a >

C

Cu

S-i



Unrelated

Cousin

Half sibling

Full sibling

Relationship to nestlings

White-fronted bee-eaters are African birds that nest colonially (Figure 53.10). Most breeding pairs are assisted by nonbreeding adults that help incubate their eggs and feed nestlings. Nearly all of these helpers assist close relatives. When helpers have a choice of two nests at which to help, about 95 percent of the time they choose the nest with the young most closely related to them.

Several other pieces of evidence suggest that the helping behavior of white-fronted bee-eaters evolved through kin selection. First, both males and females help to care for nestlings, but males help more often than females. Males remain in the social group in which they were born, but females join other social groups when they mature. Therefore, females typically live in

social groups composed primarily of nonreproductive s.

Second, individuals do not appear to gain anything other than inclusive fitness by helping—helpers are not, for example, more successful when they become breeders themselves than birds that do not help. Finally, nests with helpers produce more fledglings than do nests without helpers, showing that helpers do increase the number of fledglings produced by their close relatives. Notice that all these patterns are consistent with the principle that bee-eaters behave in ways that improve their fitness, not in

ways that increase their reproductive success or relatives' reproductive success, ways that benefit the altruist's own reproductive success, ways that benefit the altruist's relatives' reproductive success.

Species whose social groups include sterile individuals

tive success.

Many social groups consist of some individuals that are

close relatives and others that are unrelated or distantly related. Individuals of some species recognize their relatives and adjust their behavior accordingly. White-fronted bee-

is said to be eusocial. This extreme form of social behavior—the "ultimate altruism"—has evolved in termites and

In these

ultimate altruism, many hymenopterans (ants, bees, and wasps), species, worker females defend the group against predators



Eaton burchelli

53.11 Sterile Individuals are Extreme Altruists

Eusocial insect species contain classes of sterile individuals. These soldier ants from Panama protect their nests and nestmates with their large, powerful jaws.

*

or bring food to the colony, but do not reproduce. Some species have soldiers with large defensive weapons (Figure 53.11). These workers are at risk of being killed while defending the colony.

Both genetic and environmental factors facilitate the evolution of eusociality. The more closely related individuals are to one another, the greater the advantages they can receive by forgoing their own reproduction to help relatives reproduce. The British evolutionary biologist W. D. Hamilton first suggested that eusociality evolved among ants, bees, and wasps because members of the order Hymenoptera have an unusual sex determination system in which males are haploid but females are diploid.

Among the Hymenoptera, a fertilized egg hatches into a female; an unfertilized egg hatches into a male. If a female copulates with only one male, all the sperm she receives are identical because the haploid males have only one set of chromosomes, all of which are transmitted to each sperm cell. Therefore, a female's daughters share all of their father's genes. They also share, on average, half of the genes they receive from their mother. As a result, on average they share 75 percent of their alleles rather than 50 percent, as they would if both parents were diploid. Workers therefore can increase their fitness more by helping their sisters than by reproducing themselves, because they are genetically more similar to their sisters than they would be to their own offspring.

Mating between close relatives, known as inbreeding, can also generate close genetic relationships. Even if two mates are unrelated, but each is the product of generations of intense inbreeding, their offspring can be genetically nearly identical. These individuals would also benefit from helping to rear siblings. Genetic similarity generated by inbreeding could explain the evolution of eusociality among the many hymenopteran species in which queens mate with many males and among termites and naked mole-rats—the most extremely eusocial mammals—in which both sexes are diploid.

BEHAVIORAL ECOLOGY 955

Eusociality may also be favored if establishment of new colonies is difficult and dangerous. Nearly all eusocial animals construct elaborate nests or burrow systems within which their offspring are reared (Figure 53.12). Naked mole-rats live in underground colonies containing 70 to 80 individuals. The tunnel systems are maintained by sterile workers. Breeding is

restricted to a single queen and several kings that live in a nest chamber in the center of the colony. Individuals attempting to found new colonies are at high risk of being captured by predators, and most founding events fail. Thus, high predation rates, which favor cooperation among founding individuals, may facilitate the evolution of eusociality.

Unrelated individuals may behave altruistically toward one another

It is easy to understand how altruistic behavior can evolve among related individuals. It is more difficult to explain the existence of warnings of danger, sharing of food, and grooming among unrelated individuals of the same species, or even between members of different species. How can we explain the evolution of such behavior?

Such behavior among unrelated individuals could evolve through reciprocal altruism; that is, if helpers are in turn recipients of beneficial acts by the individuals they have helped. If there is a genetic basis for the acts, natural selection may increase the frequency of alleles governing this behavior. In order for reciprocal altruism to be a force, several social conditions must be present:

- ▶ Individuals in the group must know one another.
- ▶ They must associate for long periods.
- ▶ Individuals must be aware that their altruistic acts are



53.12 Termite Mounds are Large and Complex

Immense termite mounds such as this one in Kenya are costly to construct and maintain. Elaborate nests or burrows are characteristic of nearly all eusocial animals.

956 CHAPTER FIFTY-THREE

Reciprocal altruism is especially highly developed among rYuman[^], in which these conditions prevail. It illustrates the subtle adjustments in behavior among individuals in social groups.

The Evolution of Animal Societies

The decisions animals make about where to live, with whom to mate, and whether and how to care for their offspring all help determine the type of social system they have. Today's social systems are the result of long periods of evolution, but there are few records of past social systems because behavior leaves few traces in the fossil record. Possible routes of the evolution of social systems must therefore be inferred primarily from current patterns of social organization. Fortunately, many stages of social system complexity exist among species, and the simpler systems provide clues about the stages through which the more complex ones may have passed.

Parents of many species care for their offspring

The origins of all animal societies lie in the association of parents with their offspring. Individuals of many species invest time and energy in caring for offspring. Parental care increases the chances of an offspring's survival, but it usually reduces the ability of the parent to produce additional

offspring.

Parental care may also lower the chances of survival of the parent itself, because the parent could have used the time and energy to engage in other activities that would improve its own chances of survival. In other words, parents balance trade-offs between the success of their current offspring and their own future survival and reproduction.

Males and females often differ strikingly in the kinds and amounts of parental investment they can and do make. Birds, mammals, and fishes illustrate these differences and why they exist. Only female mammals have functional ^wrtfe mma.rv glancJs^nc^pSrannt p rodncp m ilk. Therefore parental care among mammals is usually given by females. On the other hand, among birds, all aspects of reproduction except production of eggs and sperm can be performed readily by both males and females. Not surprisingly, both males and females feed their offspring in about 90 percent of bird species.

Sex roles among fishes differ from those of birds and mammals because most fish species do not feed their young. Parental care consists primarily of guarding eggs and young from predators (Figure 53.13). In many fish species, males are the primary guardians. A male can guard a clutch of eggs while attracting additional females to lay eggs in his nest. A female, on the other hand, can produce another clutch of eggs sooner if she resumes foraging immediately after mating than if she spends time guarding her eggs.

The most widespread form of social system is the family, an association of one or more adults and their dependent offspring. If parental care lasts a long time, or if the breeding season is longer than the time it takes for offspring to

f

*Vn



Abudefduf saxatilis

53.13 A Sergeant Major Guards His Young

This male is defending the eggs a female has deposited. He can court other females while guarding the eggs.

mature, adults may still be caring for younger of fspring when older offspring reach the age at whic h they c ould help their parents.

"Many communal breeding systems, such as the white-fronted bee-eater families described above, most likely evolved via the extended family route. Most mammals evolved social behavior by this route. In simple mammalian social systems, solitary females or male-female pairs care for their young. In species whose young require a long period of parental care, o lder offsp ring are still prp spnt wjien the next generation is bo rru_ and they oft en help rear their younger siblings. In most social mammal species, female offspring re main in the group in which they w ere born, but males tend to _leav e—or are driven out—and must seek other soc i al grou ps. Therefore, among mammals, most helpers are females.

53.14 Savanna Weaverbirds Nest Colonially

Many African weaverbirds nest in colonies in isolated trees. Although these nests are highly conspicuous, it is difficult for most predators to get to them at the tips of the small, thorny branches.



(a) *Coiuwchaetes taurinus*

53. 15 Cooperation among Florida Scrub Jays

These Florida scrub jay helpers, most of which are offspring from the previous breeding season, are helping to feed nestlings and defend the nest against predators, such as the approaching snake. By doing so, they are improving their inclusive fitness.

The environment influences the evolution

of animal societies >-*~Q> *^

The type of s ocial organizati on a species evolves is strongly relate d to the environment in which it lives. Among the N weaverbirds of Africa, species that live i n fqr ests eat insects, J feed alone, and build well-hidden nests. Most of these

species are monogamous, and males and females look alike. In

marked contrast, weaverbirds that live in tree-studded grasslands called savannas eat primarily seeds, feed in large flocks, and nest in colonies, usually in isolated Acacia trees where their nests are large and conspicuous (Figure 53.14). In most colonial species, males have several mates and are more brightly colored than females.

These striking differences probably evolved because nesting sites and food in forests are common and widely dispersed. Solitary pairs can use these resources more efficiently than animals in groups can. In savannas, however, good nesting trees are scarce and highly clumped. Males compete for these limited nest sites; males that hold the best sites—near the tips of branches where they are safe from predators—attract the most females. Males spend their time attempting to attract additional mates rather than helping to rear the offspring they already have, which explains the evolution of brighter plumage among males.

Florida scrub jays live all year on territories, each of which is home to a breeding pair and up to six helpers

BEHAVIORAL ECOLOGY 957

(Figure 53.15). Nearly all the helpers are offspring from the previous breeding season that remain with their parents. This social system probably evolved because all suitable territories are occupied, and young individuals have little chance of establishing new territories on their own. By staying in their parents' territory and helping to raise their siblings, they both improve their inclusive fitness and have a chance of taking over the territory if one of their parents dies. Among the herbivorous hoofed mammals of Africa, social organization and feeding ecology are correlated with the diet of the animals. Smaller animals have higher metabolic demands per unit of body weight than do larger ones. Therefore, smaller hoofed mammals feed preferentially on high-protein foods such as buds, young leaves, and fruits. These foods are dispersed throughout forests, which also provide cover in which to hide from predators. Hiding is a tactic that is effective for solitary animals. In contrast, the largest hoofed mammal species are able to eat lower-quality food, but they must process great quantities of it each day. They feed in grasslands with abundant herbaceous vegetation, follow the rains to areas where grass growth is best, and live in large herds (Figure 53.16a).

Among primates, many diurnal species will eat insects and other animal food when they are available, but most of

(b) *Papio cynocephalus*

>:- " ** % ~*'-^ lte% *m\

s - .



53.16 Many Mammals of Open Country Live in Large Groups

(a) East African wildebeest live in large herds that follow the rains to places with fresh grass. (b) Baboons are conspicuous as they forage but are seldom attacked because the formidable males cooperate in defending the group.

958 CHAPTER FIFTY-THREE

them eat fruits, seeds, and leaves. In Africa and Asia, primate group sizes are smallest among arboreal forest-dwelling species, whatever their diets, and largest among the ground-dwelling savanna species, such as baboons (Figure 53.16b). Troops of foraging baboons are conspicuous to predators, but their large males help protect the other troop members. Baboons have a complex social system. In troops with more than one male, strong dominance hierarchies exist among the males, and one or two of them father most of the offspring. Females may also have dominance relationships, and young females often assume the status of their mothers when they mature.



Chapter Summary

- Ecologists study the nature and consequences of interactions among organisms and their environments.
- Behavioral ecology is the study of how animals decide where to carry out different activities, select the resources they need, respond to predators and competitors, and interact with conspecifics.

Balancing Costs and Benefits of Behaviors

- ▶ Cost-benefit analyses of behavior are based on the principle that animals have only limited amounts of time and energy to devote to their activities.
- ▶ A cost of defending a territory may be increased risk of mortality. Review Figure 53.1

Choosing Where to Live and Forage

- ▶ Selecting a habitat in which to live is one of the most important decisions an individual makes.
- ▶ The cues animals use to select habitats are good predictors of conditions suitable for future survival and reproduction.
- ▶ Foraging theory was developed to understand how animals select prey. Review Figure 53.2

Mating Tactics and Roles

- ▶ Individuals choose their associates, how to interact with them, and when to leave them. The most important choice of associates is the choice of a mate.
- ▶ Because males produce enough sperm to fertilize many eggs, males typically increase their reproductive success by mating with many females. The reproductive success of females is typically limited by the cost of producing eggs. As a result, males usually initiate courtship and often fight for opportunities to mate with females. Females seldom fight over males and often reject courting males.
- ▶ Sexual selection often leads to exaggerated traits. Review Figure 53.3
- ▶ While courting, males signal their desirability as mating partners and may perform behaviors that increase the probability that their sperm will fertilize eggs.
- ▶ Males of some species defend territories that contain food, nesting sites, or other resources. Review Figure 53.4
- ▶ By paying particular attention to those signals at which males cannot cheat, females have favored the evolution of "reliable" signals of mate quality.
- ▶ DNA fingerprinting methods have shown that social fathers often are not genetic fathers.

Costs and Benefits of Social Behavior

- ▶ Benefits of social living include better opportunities to capture prey and to avoid predators. Review Figure 53.8
- ▶ Costs of social living include competition for food, interference by conspecifics, and transmission of diseases.

Categories of Social Acts

- ▶ Acts performed by individuals living together can be grouped into four descriptive categories: altruistic, selfish, cooperative, and spiteful. Review Figure 53.9
- ▶ Altruism among closely related individuals can evolve by means of kin selection because individuals that help close relatives can improve their inclusive fitness. Review Figure 53.10
- ▶ Eusocial systems with sterile individuals have evolved among termites, hymenopterans (ants, bees, and wasps), and in a mammal, the naked mole-rat.

The Evolution of Animal Societies

- ▶ The origin of most animal societies is the family, an association of one or more adults and their dependent offspring.
- ▶ The type of social organization a species evolves is strongly related to the environment in which it lives.

For Discussion

1. Most hawks are solitary hunters. Swallows often hunt in groups. What are some plausible explanations for this difference? How could you test your ideas?
2. Because costs and benefits of behaviors can seldom be measured directly, behavioral ecologists often use indirect measures such as correlations between behavior patterns and the presence of predators. What are the strengths and weaknesses of some of these indirect measures?
3. Polyandry is a mating system in which one female has a "harem" of several males. Why is polyandry much rarer among both birds and mammals than polygyny, the situation in which one male forms pair bonds with several females?
4. When frogs mate, a male clasps a gravid female behind

her front legs and stays with her until she lays her eggs, at which time he fertilizes them. In most species of frogs, the male remains clasped to the female for a short time, usually no longer than a few hours. However, in some species, pairs may

remain together for up to several weeks. In view of the fact that a male cannot court or mate with any other female while clasping one, and that a female lays only a single clutch of eggs, why is it advantageous for males to behave this way? What can you guess about the breeding ecology of frogs that remain clasped for long periods? Why should females permit males to clasp them for so long? (Females do not struggle!)

5. Among vertebrates, helpers are individuals capable of reproducing, and most of them later breed on their own. Among eusocial insects, sterile castes have evolved repeatedly. What differences between vertebrates and insects might explain the failure of sterile castes to evolve in the former?

6. The use of DNA fingerprinting technology has shown that in many species, social partners and genetic partners differ. Under what conditions do individuals benefit from copulating with individuals other than their social mates? Do males and females benefit equally from this behavior?



Population Ecology



Large saguaro cacti are conspicuous features of the Sonoran Desert in southern Arizona. But finding a seedling cactus is difficult—at least, until you learn where to search. All the small cacti are found beneath trees or shrubs.

In the harsh environment of the Sonoran Desert, plants are exposed to intense daytime heat and wide temperature fluctuations. Their roots are in extremely dry soil much of the year. Small plants are most vulnerable to these conditions because they have small root systems, and daytime temperatures are extremely high at the soil surface. Therefore, although seeds of saguaro cacti are dispersed widely over the desert by birds, seedling cacti survive only in the shade of trees and shrubs, called nurse plants, where they are protected from the intense daytime heat. Thus, the density and distribution of saguaro cacti are strongly influenced by the number and location of trees and shrubs.

All the individuals of saguaro cacti—or of any other species—within a given area constitute a population. The sizes of populations continually change. To understand how and why these changes happen, population ecologists count individuals in different locations and try to determine the factors that influence birth, death, immigration, and emigration rates.

In addition to studying the dynamics of populations in a particular area, population ecologists also investigate changes over the entire ranges of species. They attempt to answer questions such as: What causes a species to be common or rare? Why is a species common in some parts of its geographic range and rare in others? What determines the limits of the ranges of species?

In this chapter we discuss how and why the sizes of populations vary over space and time, and show how this ecological knowledge is used to predict and manage the growth of populations. To set the stage for studies of populations, we present some background information on how the individuals of a population are distributed.

Population Structure: Patterns in Space and Time

At any given moment, an individual organism occupies only one spot and is of one particular age. The members of

A Cactus Needs Shade

This saguaro cactus (*Cereus giganteus*) grew in the shade of the palo verde tree, which protected it from the intense heat.

A population, however, are distributed over space and differ in age and size. These features are among the components of population structure. As we saw in Chapter 21, geneticists and evolutionary biologists also study population structure, but they are interested primarily in distributions of genotypes and their degree of isolation from one another, because that component of population structure influences how populations evolve.

Ecologists study population structure at different spatial scales, ranging from local subpopulations to entire species. They study the numbers and spatial distributions of individuals because these features influence the stability of populations and affect interactions among species.

Density is an important feature of populations

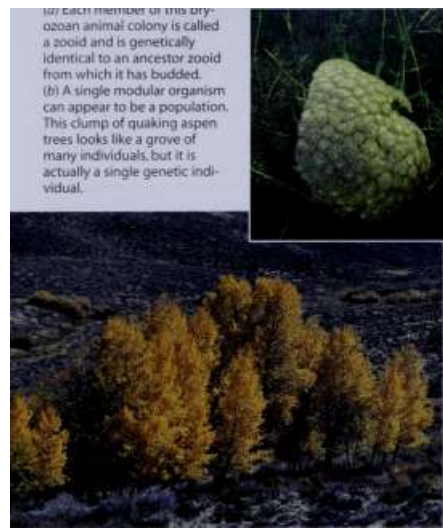
The number of individuals of a species per unit of area (or volume) is its population density. Ecologists are interested in population densities because dense populations often

(a) Each member of this bryozoan animal colony is called a zooid and is genetically identical to an ancestor zooid from which

it has budded.

(b) A single modular organism can appear to be a population. This clump of quaking aspen trees looks like a grove of many individuals, but it is actually a single genetic individual.

(a) *Pectinatella magnified*



(b) *Populus tremuloides*

exert strong influences on their own members as well as on populations of other species. Other scientists—such as those working in agriculture, conservation, or medicine— wish to manage species to raise (in the case of crop plants, aesthetically attractive species, or threatened or endangered species) or lower (in the case of agricultural pests and disease organisms) their densities . To manipulate population densities, we must know what factors make populations increase and decrease in size, and how those factors work.

Because species and their environments differ , population densities are measured in more than one way. Ecologists usually measure the densities of organisms in terrestrial environments by the number of individuals per unit of area, but number per unit of volume is generally a more useful measure for organisms living in water. For species whose members differ markedly in size, as do most plants and some animals (such as mollusks, figeivand reptiles), the total mass of individuals — the biomass—may be a more useful measure of density than the number of individuals.

The fertilized egg of many organisms develops into a zygote of construction called module, which produces addi-

tional modules much like itself. Many plants are modular, and there are many groups of modular protists, fungi, and animals (for example, sponges, corals, and bryozoans; Figure 54.1a). A modular organism may grow to a large size, and it is often difficult to distinguish a modular organism

from a cluster of genetically separate individuals (Figure 54.1b). The effects of modular organisms on their environment often depend primarily on the number and size of the modules. Therefore, ecologists studying modular organisms are often concerned primarily with the number, size, and shape of the modules rather than with the number of genetically distinct individuals.

Under some circumstances, the individuals in a population can be counted directly without missing any of them or counting any of them twice, but this process is usually impossible or too laborious. Ecologists commonly estimate population densities by sampling a population in a representative area and extending their findings to a larger area.

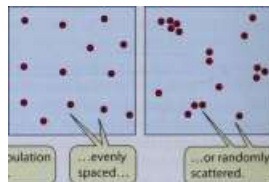
The size of a population can also be estimated by marking and recapturing individuals. For example, if we capture and mark 100 individuals in a population, we can take another sample later and count the individuals in that sample that are already marked. If, say, 10 percent of the individuals in our second sample are already marked, we would conclude that the population contains about 1,000 individuals.

This estimate is based on the mathematical assumption that the number of individuals caught the first time is the same proportion of the total population as the proportion

-sXX-

(a)

Individuals in a population may be clumped...



..evenly spaced...



■ »'---'-MC;; ■ •- «ri

<m:

(b)

54.2 Patterns of Spatial Distribution

(a) A diagrammatic representation of clumped, even, and random distribution patterns, (b) The relatively even spacing of these Australian desert plants results because each established plant removes so much water from the surrounding soil that no young plants can grow within its root zone.

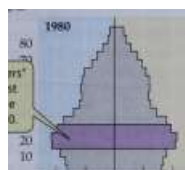


(b)

(c)

"Baby boom age group

| "Baby boomers" were the most dominant age group in 1980.



Children of "baby boomers"

80 70 60 50 40 30

-29.

10

80 70 60 50 40

2020

£

Q By 2020, the children of "baby boomers" will be as dominant as their parents.

i

10 5 0 5 10 Millions of persons

1870

i—

1880 1890 1900

Year 1910 1920 1930

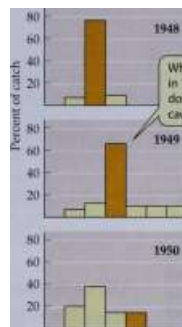
1940 1950 1960

i r

In 1971, when these data were collected, the population was dominated by trees that were recruited (began growing) between 1931 and 1942.

L

60 40



s

3

c

>

04

Whitefish hatched in 1944 were the dominant age group caught through 1949.

MI

JL L

JL

IUlhill, JL

I



2 3 4 5 Age group

6 7 (years)

tion of marked individuals to the total number caught the second time

Spacing patterns reflect interactions among individuals

Ecologists studying population structure also look at the way in which the individuals in a population are spaced. Individuals in a population may be tightly clumped together, evenly spaced, or randomly scattered (Figure 54.2a). Distributions can become clumped when young individuals remain close to their birthplaces, when suitable habitat patches are "islands" separated by unsuitable areas, or by chance. The relatively even spacing of many plants is a result of competition for light, water, and soil nutrients (Figure 54.2b). Among animals, defense of space is the most common cause of even distributions (see Figure 53.4b). Random distributions may result when many factors interact to influence where individuals settle and survive. The saguaro cacti in the Sonoran Desert (discussed at the beginning of this chapter) are distributed randomly because that is how suitable environments are distributed.

96 91 86 81 76 71 66 61 56 51 46 41 36 31 26 21 16 11 6 Age (years)

54.3 Age Distributions Are Influenced by Timing of Births

A period when birth rates are high may be reflected in a population's age structure for many years, as in (a) humans in the

United States, (6) whitefish in Lake Erie, and (c) black cherry trees in a Wisconsin woods.

Age distributions reflect past events

Populations are composed of individuals ranging from newborns to postreproductive adults. In this chapter we consider an individual to be "born" when it leaves its mother's body as a seed, egg, or baby. The proportions of individuals in each age group in a population make up its age distribution. The density and spacing of individuals are spatial attributes of a population; age distribution is a temporal (time-oriented) attribute.

The timing and rates of births and deaths determine age

distributions. If both birth rates and death rates are high, a

population will be dominated by young individuals. If birth rates and death rates are low, a relatively even distribution of individuals of different ages results. The age distribution of a population thus reveals much about its past history of births and deaths.

The timing of births and deaths may influence age distributions for many years in populations of long-lived species. The human population of the United States is a good example. Between 1947 and 1964, the United States experienced what is called the post-World War II "baby boom." During these years, average family size grew from 2.5 to 3.8 children; an unprecedented 4.3 million babies were born in 1957. Birth rates declined during the 1960s, but Americans born during the baby boom will constitute the dominant age class into the twenty-first century (Figure 54.3a). "Baby boomers" became parents in the 1980s, producing another bulge in the age distribution—a baby boom

The timing of births and deaths may influence age distributions for many years in populations of long-lived species. The human population of the United States is a good example. Between 1947 and 1964, the United States experienced what is called the post-World War II "baby boom." During these years, average family size grew from 2.5 to 3.8 children; an unprecedented 4.3 million babies were born in 1957. Birth rates declined during the 1960s, but Americans born during the baby boom will constitute the dominant age class into the twenty-first century (Figure 54.3a). "Baby boomers" became parents in the 1980s, producing another bulge in the age distribution—a baby boom

962 CHAPTER FIFTY-FOUR

The number of individuals in a population at any given time is equal to the number present at some time in the past, plus the number born between then and now, minus the number that died, plus the number that immigrated, minus the number that emigrated. That is, the number of individuals at a given time, N_t is given by the equation

$$N_t = N_0 + B - D + I - E$$

Survivorship = the proportion of newborns who survive to age x .

Survival rate = the proportion of individuals of age x who survive to age $x + 1$.

Mortality rate = the proportion of individuals of age x who die before the age of $x + 1$.

"echo"—but they had, on average, fewer children than their parents, so the bulge is not as large. Similarly, in Lake Erie, 1944 was such an excellent year for reproduction and survival of whitefish that individuals of that age group dominated whitefish catches in the lake for several years (Figure 54.3b). The population of black cherry trees in a Wisconsin woods is dominated by individuals that began growth between 1923 and 1941 (Figure 54.3c).

Population Dynamics: Changes over Time

At any moment in time, a population has a particular structure determined by the number and distribution of its members in space and their ages. However, as we have just seen, population structure is not static. Changes in the structure of a population influence whether it will increase or decrease; that is, they affect the dynamics of a population. We will now examine how ecologists measure birth and death rates and use that information to understand how population densities change. The study of changes in the size and structure of populations is known as demography.

Births, deaths, and movements drive population dynamics

Knowledge of when individuals are born and when they die provides a surprising amount of information about a population. Births, deaths, and movements of individuals are demographic events—that is, they determine the numbers of individuals in a population. Ecologists measure the rates at which these events take place—the number of such events per unit of time. These rates are influenced by environmental factors, the life history traits of the species, and by population density.

where N_1 is the number of individuals at time 1; N_0 is the number of individuals at time 0; B is the number of individuals born, D the number that died, I the number that immigrated, and E the number that emigrated between time 0 and time 1. If we measure these rates over many time intervals, we can determine how a population's density changes.

Life tables summarize patterns of births and deaths

Life tables summarize data about births and deaths that

can be used to predict future growth rates of populations. We can construct a life table by determining for a group of individuals born at the same time—called a cohort—the number still alive at specific times and the number of offspring they produced during each time interval. An example, based on an intensive study of one population of Darwin's finches carried out on Isla Daphne in the Galapagos archipelago, is shown in Table 54.1.

The data in Table 54.1 are based on a cohort of 210 birds that hatched in 1978 and were followed until 1991, by which time all of them had died. This life table (which presents data only on survival, not on reproduction) shows that the mortality rate was

as high during the first year of life. It then dropped dramatically for several years, followed by a general increase in later years. Mortality rates fluctuated among years because survival of the birds depends on seed production, which is strongly correlated with rainfall. The Galapagos archipelago experiences both drought years, during which plants produce few seeds, birds do not nest, and adult survival is poor, and years of heavy rainfall, during which seed production is high, most birds breed several times, and adult survival is high. The life table reflects these fluctuations.

Ecologists often use graphs to highlight the most important changes in birth and death rates in populations. Graphs of survivorship—the mirror image of mortality—in relation to age show at what ages individuals survive well and at what ages they do not. To interpret survivorship data, ecologists have found it useful to compare real data with several hypothetical curves that illustrate a range of

possible survivorship patterns (Figure 54.4f). At one extreme, nearly all individuals survive for their entire potential life span and die at about the same age (hypothetical curve I). At the other extreme, the survivorship of young individuals is very low, but survivorship is high for most of the remainder of the life span (hypothetical curve III). An intermediate possibility is that survivorship is the same throughout the life span (hypothetical curve II).

Survivorship data from real populations often resemble one of these hypothetical curves. For example, survivorship of humans in the United States remains high for many decades but then declines significantly in older individuals (Figure 54.4g). Many wild birds have survivorship curves similar to hypothetical curve I; the probability of their surviving is about the same over most of the life span once they are a few months old (Figure 54.4c).

A widespread survivorship pattern is found among organisms that produce many offspring, each of which receives few energy resources and no parental care. In these species, low survivorship of young individuals is followed by high survivorship during the middle part of the life span, and then low survivorship toward the end of the life

span. *Spergula vernalis*, an annual plant that grows on sand dunes in Poland, illustrates this pattern (Figure 54.4d).

n



Patterns of Population Growth

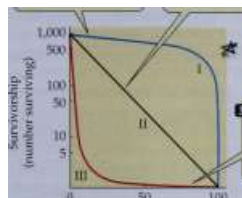
If a single bacterium selected at random from the surface of this book, and all its descendants, were able to grow and reproduce in an unlimited environment, explosive population growth would result. In a month the bacterial colony would weigh more than the visible universe and would be expanding outward at the speed of light. Similarly, a single pair of Atlantic cod and their descendants, reproducing at the maximum rate of which they are capable, would fill the Atlantic Ocean in 6 years. But, as Darwin observed, this does not happen in nature.

All populations have the potential for explosive growth because as the number of individuals in the population increases, the number of new individuals added per unit of time accelerates, even if the rate per capita of population increase remains constant. This form of explosive increase is called exponential growth. If we ignore immigration and emigration and assume that births and deaths occur continuously and at constant rates, such a growth pattern forms a continuous curve (Figure 54.5a). This curve can be expressed mathematically in the following way:

(a) Hypothetical curves

I Most individuals may survive their potential lifespan, then die.

| Survivorship may be the same throughout the lifespan...



...or survivorship may be low for the young and remain high for the rest of the life span.

50 100

Percent of life span

Rate of increase in number of individuals 'Average per capita birth rate λ - Average per capita death rate

x Number of individuals

or, more concisely,

$r =$

$\frac{\Delta N}{\Delta t}$

$= (b-d)N$

where $\Delta N/\Delta t$ is the rate of change in the size of the population (ΔN = change in number of individuals; Δt = change in time). The difference between the average per capita birth rate (b) and the average per capita death rate (d) is the per capita rate of increase (r). In these equations

(b)

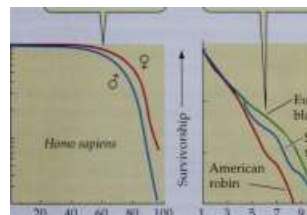
(c)

(d)

3

Human survivorship resembles curve I.

Wild bird survivorship resembles curve II.



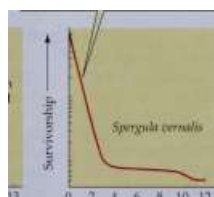
Survivorship in annual plants—with many offspring and no parental care—resembles curve III.

European blackbird

Song thrush

20 40 60 80 Age in years

3 5 7 9 11 Age in years

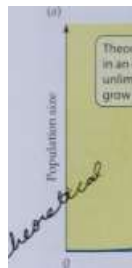


4 6 8 10 12 Age in months



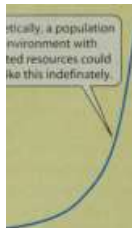
54.4 Survivorship Curves

Survivorship curves show the number of individuals in a cohort still alive at different times over the life span, (a) The range of possible survivorship patterns. Patterns for (b) humans in the United States, (c) some small wild birds, and (d) an annual plant, *Spargula vernalis*, on Polish sand dunes.

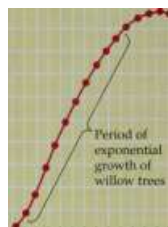


(b)

Theoretically, a population in an environment with unlimited resources could grow like this indefinitely.

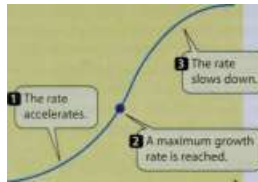


500
 400
 300 -
 C
 200
 01
 £
 -
 Z
 100
 (o
 Time
 Carrying capacity of environment (K)
 MM •
 1966



actual

Period of exponential growth of willow trees
 1970
 1975 Year
 1980
 1984
 rn



A maximum growth rate is reached.

Time

both births and immigration and d includes both deaths and emigration.

When there are no limits on population growth, r has its highest value, called r_{max} . The r_{max} has a characteristic value for each species. Therefore, the rate of growth of a population under optimal conditions is

$\frac{dN}{dt} = r_{max} N$

N

54.5 Exponential and Logistic Population Growth

(a) A theoretical exponential growth curve. (b) Growth curve of an actual population of willows at Newborough Warren, Wales. The trees experienced a surge of exponential growth when rabbits that fed on the leaves were decimated by disease, (c) a population in an environment with limited resources usually stops growing exponentially long before it reaches the environmental carrying capacity.

The simplest way to picture the limits imposed by the environment is to assume that it can support no more than a certain number of individuals of a particular species. This number, called the carrying capacity of the environment, is determined by the availability of resources—food, nest sites, shelter—as well as by disease, predators, and, in some cases, social interactions. Rather than being exponential, population growth slows down as the population approaches the carrying capacity, so that the growth curve has an S shape (Figure 54.5c).

The S-shaped growth pattern, which is characteristic of many populations growing in environments with limited resources, can be represented mathematically by adding to the equation for exponential growth a term $-\frac{N^2}{K}$, that



But optimal conditions do not continue indefinitely, and growth rates eventually slow down, as we explore in the following section.

Population growth is influenced by the carrying capacity

Natural populations may experience exponential growth for short periods of time under favorable conditions. For example, one population of willows in Wales increased dramatically in the 1970s after the rabbit population, which had severely nibbled the willows, crashed due to an outbreak of the disease myxomatosis (Figure 54.5b). But no real population can maintain exponential growth for very long because environmental limitations cause birth rates to drop

slows the population's growth as it approaches the carrying capacity (K). The simplest such equation is that for logistic growth:

$\frac{dN}{dt} = r_{max} N \left(\frac{K-N}{K} \right)$

$K-N$

K

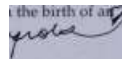
N

The biological assumption in this equation is that each individual added to the population makes things slightly worse for the others because it competes with them for available resources. Population growth stops when $N = K$ because then $(K-N) = 0$ so $(K-N)/K = 0$, and thus $dN/dt = 0$.

and decreases to rise. In fact, over long time periods, the sizes of most populations fluctuate around a relatively constant number.

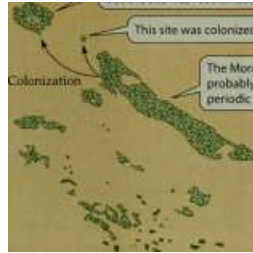
The logistic growth equation contains some important simplifications that are not true for most populations. Its most critical assumptions are that (1) each individual exerts equal effects immediately at birth; (2) all individuals produce equal effects on the population; and (3) births and deaths are independent of age. However, in nature, organisms grow during their lives, and their effects on others normally increase with age, so there may be a delay between the birth of an

individual



This population became extinct in 1976, but the site was recolonized in 1988.

This site was colonized in 1986.



The Morgan Hill population is probably the source of individuals for periodic colonization of other sites.



Bay checkerspot butterfly

10 km

H¹

^

has been studied for many years by Stanford University biologists. During drought years, most host plants die early in spring before the butterfly larvae have pupated. At least three butterfly subpopulations became extinct during a severe drought in 1975-1977. The largest patch of suitable habitat, Morgan Hill, typically supported thousands of butterflies (Figure 54.6). It probably served as a source of individuals that dispersed to and colonized small habitat patches where the butterflies had become extinct.

Population Regulation $UL^c / ^{-}$

In a limited environment, population growth slows down as density increases because the members increasingly affect one another adversely. As a result, a population above the environmental carrying capacity is likely to decrease in density, and one that is below the carrying capacity is likely



to increase. In this section we discuss how populations may

be influenced by interactions between their density and the

54.6 Subpopulation Dynamics . . . J

The population of the bay checkerspot butterfly *Euphydryas* carrying capacity of their environment.

editha bayensis is divided into a number of subpopulations con-

finned to habitat patches that contain the plants its larvae feed on. How does population density influence birth and

death rates?

individual and the time at which it begins to affect the other members of its population. A seedling tree, for example, exerts a much smaller effect on its neighbors than a large adult tree does, and it does not begin to reproduce until it reaches a relatively large size.

In addition, the logistic equation models a population in a single habitat patch; it does not take immigration and emigration into account. Next we consider the dynamics of an assembly of local populations, which is more complex than the growth of a single population.

Many species are divided into discrete subpopulations

Many populations are divided into discrete subpopulations among which some exchange of individuals occurs. Such a pattern is often found where suitable habitat occurs in separated patches, or "habitat islands." Each subpopulation has a probability of "birth" (immigration) and "death" (extinction). Within each subpopulation, growth occurs in the ways we

have just discussed, but because subpopulations are typically much smaller than the population as a whole, local disturbances and random fluctuations in numbers of individuals often cause the extinction of a subpopulation. However, if individuals frequently move between subpopulations, immigrants may prevent declining subpopulations from becoming extinct. This process is known as the rescue effect.

The bay checkerspot butterfly provides a good illustration of the dynamics of such divided populations. The larvae of this butterfly feed on only a few species of annual plants that are restricted to outcrops of a particular kind of rock on hills south of San Francisco. The bay checkerspot

If per capita birth or death rates change in response to population density, they are said to be density-dependent. Death and birth rates may be density-dependent for several reasons:

- As a population increases in abundance, it may deplete its food supply, reducing the amount of food that each individual gets. Poor nutrition may increase death rates and decrease birth rates.
- Predators may be attracted to regions where densities of their prey have increased. If predators are able to capture a larger proportion of the prey than they did when prey were scarce, the per capita rate of predation of the prey rises.
- Diseases, which may increase death rates, spread more easily in dense populations than in sparse populations.

A population whose dynamics are influenced primarily by density-dependent factors is said to be regulated.

Factors that change per capita birth and death rates in a population in dependence of its density are said to be density-dependent. A very cold spell in winter, for example, may kill a large proportion of the individuals in a population regardless of its density. However, even environmental factors whose frequency and severity are unrelated to population density may result indirectly in density-dependent mortality. Cold weather may not kill organisms directly, but may increase the amount of food individuals need to eat each day. Individuals pushed by population density into poorer foraging areas may be more likely to die than those in better foraging areas. Or the death rate may be related to the quality of sleeping places. If population density is high, a larger proportion of individuals may be forced to sleep in places that expose them to the cold.

Various combinations of density-dependent and density-independent factors can influence the density of a popula-

966 CHAPTER FIFTY-FOUR

Equilibrium is reached when birth rates and death rates are equal.

If birth rate or death rate, or both, are density-dependent, a population's size tends to fluctuate around an equilibrium value.

Density-dependent, birth rate

Equilibrium density

Density-independent death rate

Density-dependent birth rate

Density-dependent death rate



Equilibrium density

Population density

Population density

Population density

m



t

54.7 Density-Dependent Factors Regulate Population Size

The densities of all populations fluctuate, but they tend to return to equilibrium value if either birth rate and/or death rate are density-dependent.

tion. The hypothetical graphs in Figure 54.7 show how birth and death rates can change in relation to population density. When birth and death rates are equal (the point at which the two lines cross) the population neither grows nor shrinks. If

birth or death rates, or both, are density-dependent, the population responds to increases or decreases in its

population by increasing or decreasing its birth or death rates. If neither rate is density-dependent, there is no equilibrium and the population is not regulated.

Fluctuations in the density of a species' population are determined by all the factors and processes, density-dependent and density-independent, acting upon it. The combined action of density-independent and density-dependent factors is illustrated by the dynamics of a population of song sparrows on Mandarte Island, off the coast of British Columbia, Canada.

During recent years, in response to variable winter weather and other physical factors, the number of song sparrows on Mandarte has fluctuated between 4 and 72 breeding females and between 9 and 100 breeding males. Density-independent variation caused by weather is modulated by several density-dependent factors. The number of breeding males is limited by territorial behavior: The larger the number of males, the larger the number that fail to gain territories, so more live as "floaters" (Figure 54.8a). Also, the larger the number of breeding females, the fewer offspring each female fledges (Figure 54.8b). And, finally, the more offspring are fledged, the more poorly they survive over the winter (Figure 54.8c).

Disturbances affect population densities

Disturbances—short-term events that influence populations by changing their environment and, hence, its carrying capacity—regularly affect population densities. Common physical disturbances are fires, hurricanes, ice storms,

wind storms, floods, landslides, and lava flows. Biological disturbances include tree falls, disease epidemics, and the burrowing and trampling activities of animals. Disturbances differ in their spatial distribution, frequency, predictability, and severity.

A disturbance typically decreases the environmental carrying capacity for some populations but for others. A landslide, for example, may increase the carrying capacity of the environment for plants that require bare mineral soil for the germination of their seeds and survival of their seedlings. However, it will decrease the carrying capacity for species that require shade and rich organic litter for successful germination.

Populations themselves can influence the frequency of some disturbances. Immediately after a fire, for example, there is not enough combustible organic matter to carry another fire. However, as vegetation grows back, dead wood, branches, and leaves accumulate, gradually increasing the supply of fuel. Thus the frequency of fires may be proportional to the rate at which fuel accumulates through the growth of plant populations, or the rate at which herbivores consume plant materials that would otherwise accumulate. Similarly, as many trees age, their roots and trunks become weakened by fungal infections. Old, large trees are thus susceptible to being toppled by high winds. Therefore, the likelihood of a major blowdown increases as the forest ages.

Organisms cope with environmental changes by dispersing

A common response of animals to environmental changes is dispersal—movement to another habitat. If habitat quality declines greatly, individuals may be able to improve their survival and reproductive success by going elsewhere. If repeated seasonal changes alter a habitat, organisms may evolve life cycles that appear to anticipate the changes. Migration—regular seasonal movement from one place to another—is most widespread among birds, but some insects, such as monarch butterflies, and some mammals also

<2^

(a)

40 r

30 -

0 0>

* E 90

0> ac 20

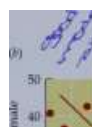
« 0

1 S

<y

I* OJ

10



K. 30

-a

01

"S 20

53

0

10

When there are more females, fewer chicks per female are fledged.

(c)



0.6

6C C

> 0.3 -

0 !«

5, .2

0 •=

£ S 0.2

3

J_

The higher the density of birds in the autumn, the poorer are the chances of surviving the winter.



_L

20 40 60 80

Total number of males

20 40 60 80

Number of breeding females

40 80 120 160

Number of adults in autumn

54.8 Regulation of an Island Population of Song Sparrows

The number of song sparrows on Mandarte Island is mainly determined by the severity of winter weather, but the weather's effect is modulated by several density-dependent factors, including (a) male territorial behavior, (b) the reproductive success of females, and (c) the survival of juveniles.

migrate (Figure 54.9). Most insectivorous birds leave high latitudes for more favorable wintering grounds in autumn, before conditions seriously deteriorate. In arctic regions, caribou migrate each year between winter and summer ranges.

its life, an individual organism ingests nutrients or food, grows, interacts with other individuals of the same and other species, reproduces, and usually moves or is moved so that it does not die exactly where it was born. Life histories describe how an organism divides its efforts among these activities. In previous discussions we have referred to various components of the life histories of organisms. Now we focus our attention specifically on life history patterns and why they have evolved to be as

variable as they are.

The life history traits of organisms have been molded by natural selection acting over many generations. In each lineage, those traits that maximized reproductive success were selected. Over time, natural selection has not produced a single,

Life Histories Influence Population Growth

reproductive pattern of reproduction. Some organisms such

The complete life history of an organism consists of its life span, usually give birth to a single offspring with growth to maturity and death. During its life span in each reproductive episode; others produce thou-

(a) *Danaus plexippus*



(b) *Rangifer tarandus*

54.9 Animals Migrate to Remain in Suitable Environments

(a) Most of North America's monarch butterflies migrate to central Mexico, where they aggregate on conifers in cool mountain valleys. They can survive the winter there without eating because their metabolic rates are low.

(b) These caribou in the American Arctic are migrating from open tundra to their winter foraging grounds at the edge of boreal forest. During the winter they feed on lichens on branches of trees.

968 CHAPTER FIFTY-FOUR

sands or millions of eggs or seeds in one bout of reproduction. Some organisms begin to reproduce within days or weeks of being born; others live for many years before reproducing. All life histories are based on a certain set of traits, which includes:

- Size and energy supply of the individual at birth
- Its rate and pattern of growth and development
- How many times individuals disperse
- The number and timing of reproductive events
- The number, size, and sex ratio of offspring
- The ages at which individuals die

Life histories include stages for growth, change in form, and dispersal

For at least part of their lives, all organisms grow by gathering and assimilating energy and nutrients. Some organisms, such as birds and mammals, gather energy and nutrients throughout their lives, even after they reach adult size and stop growing.

Energy gathered after growth stops maintains the organism and supports reproduction. In many species, however, energy gathering is confined to a particular life stage. Most moths, for example, feed only when they are larvae. The adults lack mouthparts and digestive tracts, live on the energy they gathered as larvae, and survive only long enough to disperse, mate, and lay eggs (Figure 54.10).

Individuals of many species also change form during their lives. Human babies are unmistakably human, but newborns of many species differ dramatically from adults. Some of the most striking changes are found among insects such as beetles, flies, moths, butterflies, and bees, which undergo metamorphosis from their larval to their adult forms. Many plants have resting stages, such as spores and seeds, that have low metabolic rates and are highly resistant to changes in the physical environment. Growth typically does not take place in these stages.

At some time in their lives, all organisms disperse. Some, such as plants and sessile animals, disperse as eggs, larvae, spores, or seeds. Others, such as insects and birds, disperse primarily as adults. Still others may disperse during several different life stages. Individuals of some species can change their location many times during their lives in response to environmental changes. Others remain in the first place they settle.

human dispersal by

Life histories embody trade-offs

Life history evolution is influenced by inevitable trade-offs. These trade-offs exist because changes that improve fitness by means of one life history trait often reduce fitness by means of another. What are the major tradeoffs in life history traits? One universal trade-off exists between number and size of offspring. Every newborn individual begins to grow with energy and nutrients from its maternal parent, but how much energy and nutrients individuals receive from their mothers varies greatly. The larger the amount of energy

TS>



Polyphemus sp.

54.10 A Life Stage for Sex and Reproduction Only

This female moth has no mouth or digestive tract. She will live only a few days, just long enough to mate and lay her eggs. Provided to each offspring, the larger it can grow before it must gather its own energy, but the fewer offspring a mother can produce for a given amount of energy is a major trade-off. For animals, a trade-off also exists between the number of offspring produced and the amount of care parents provide to their offspring. The more parental care the parents provide, the fewer offspring they can produce for a given investment in reproduction. Birds and mammals produce few offspring at a time and provide extensive care for each one.

Some organisms invest so much in one reproductive effort that they have no energy left for another—or even for their own survival. If two individuals have the same amount of energy to invest in reproduction, and one reproduces only once while the other reproduces several times, the former can produce more offspring in a single episode than the latter because it reserves no energy for itself. Annual plants invest nearly all of the energy they gain during their single growing season in seed production; they do not survive long after reproducing. Some short-lived organisms also reproduce once in their lifetimes and die soon afterward. Pacific salmon (genus *Oncorhynchus*) hatch in fresh water, migrate to the sea, spend a number of years at sea, return to fresh water, spawn, and die (Figure 54.11f). Most agaves (century plants) of the American Southwest likewise store up energy for many years before producing a large flowering stalk, forming many seeds, and dying (Figure 54.11b).

Trade-offs also exist between reproduction and growth. Members of many species do not begin to reproduce until they have reached full size, but others, such as most plants, mollusks, fishes, and reptiles, can reproduce while they are still relatively small and continue to reproduce as they grow. Proportionally, usually reduces growth rate because these two processes compete for the limited amount of energy an individual has at its disposal. Beech trees in Germany, for example, grew more slowly during years when they pro-



Flowering stalk

{a) *Oncorhynchus nerka*

54.11 A Single Reproductive Effort

(a) These sockeye salmon are ascending Hensen Creek, Alaska. They will lay their eggs in gravel beds in the stream and then die. (b) This century plant has mobilized the energy stored during its long life to produce a large flowering stalk with hundreds of flowers, literally reproducing itself to death.

duced large crops of nuts than they did during years when their nut crops were small (Figure 54.12).

Nonreproductive form of Agave

(b) Agave sp.

Offspring are like "money in the bank"

If reproduction compromises future growth and survival, why do some organisms start to reproduce when they are small or young? The potential contribution of an individual's offspring to future generations depends, in part, on when they are produced. A useful analogy compares the production of offspring to earning interest on money deposited in a bank. It pays to deposit money in the bank as

interest. Off-

newborn individual does not have the highest reproductive value, even though it has its full reproductive potential ahead of it, because many newborn individuals will die before they have a chance to reproduce. Therefore we must discount the number of offspring an individual could produce if it survived by the chance that it will die before reaching reproductive age, or during reproduction. When we make the appropriate calculations, we find that the reproductive value of an individual steadily increases until it begins to reproduce. Once maturity is reached, reproductive value declines; in most species, it reaches zero when the individual has finished reproducing. However, individuals can still have positive reproductive value after they have stopped reproducing that remain to be born to individuals of a particular age. An individual can continue to assist the survival of their offspring and $\frac{J}{A} < \frac{J}{A} < \frac{J}{A}$ grandoffspring.

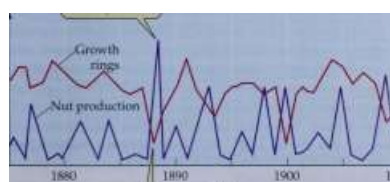
(N CrT)

soon as possible so that it can begin to earn interest produced early in an adult's life likewise "yield interest" quickly—that is, they can begin to reproduce sooner than offspring produced later. If reproductive value is the average number of offspring

In years of large nut crops..



Because reproductive value declines after maturity, the power of natural selection grows



01

1910

...growth rings typically are narrow.

Year

intensely weaker as an individual ages, once reproductive value has dropped to zero, natural selection cannot act on alleles that first produce

54.12 Reproduction Slows Growth Rates in Beech Trees

The width of annual growth rings shows the growth rate of beech trees in different years. In general, the trees grew slowly in years when they produced large crops of nuts, but during unusually favorable years they grew rapidly and produced many nuts.

970 CHAPTER FIFTY-FOUR

Contagious diseases

Pneumonia and influenza

Tuberculosis

Gastritis/enteritis/ colitis

Diphtheria

During the twentieth

century the major causes

of death in the United

States have shifted from highly contagious diseases...

1944

27.3

10.8

142.7

0.0

40.3 0.0

Typhoid/paratyphoid

Measles

31.3 0.0

113.3

0.0

1900 1985

*[.

managing populations of organisms are based on our understanding of how populations grow and are regulated.

A general principle of population dynamics is that the total number of births and the growth rates of individuals tend to be highest when a population is well below its carrying capacity. Therefore, if we want to maximize the number of individuals of a species that we wish to harvest, we should manage the population so that it is far enough below carrying capacity to have high birth and growth rates. Hunting seasons for game

Diseases of old age

Cardiovascular/renal

Cancers



345.2

422.7

64.0

192.0

100 200 300 400

Death rate per 100,000 population

500

54.13 Causes of Human Death in the United States

Today most people die of diseases of old age because improved sanitation and public water supplies, as well as medical advances such as immunization, have greatly reduced the incidence of contagious diseases that formerly killed many young people.

their phenotypic effects at that age—even those that are to the individual's survival. As a result,



highly detrimental

harmful alleles are expressed as individuals age, causing increased mortality rates, especially after reproduction has ceased. In this manner, senescence—an increased probability of dying per unit of time with increasing age—has evolved

As a result of improved hygiene and nutrition, most people in modern industrial societies are now spared the contagious diseases that cause death rates to be high among people of all ages in nonindustrial societies. Most people live to the age when the so-called genetic diseases of old age begin to afflict them. Cancer and heart disease, the main killers in industrialized societies, are much more difficult to cure than the contagious diseases that formerly caused most deaths. For this reason, despite the expenditure of enormous resources to extend life, the average age of death in the United States has changed very little during the past 100 years. As one source of mortality is eliminated, another takes its place (Figure 54.13). Life history theory suggests that this situation is likely to continue indefinitely.

Can Humans Manage Populations?

For many centuries, people have tried to reduce populations of species they consider undesirable and increase populations of desirable species. Strategies for controlling and

birds and mammals are established with this objective in mind.

...to the diseases. Life history traits determine how heavily of old age, a population can be exploited

Populations with high reproductive capacities can sustain their growth despite a high rate of harvest. In such populations (many species of fish, for example), each female may lay thousands or millions of eggs. Another characteristic of these fast-reproducing populations is that individual growth is often density-dependent. If prereproductive individuals are harvested at a high rate, the remaining individuals may grow faster. Many fish populations can be harvested heavily for many years because only a modest number of females must survive to reproductive age to produce the eggs needed to maintain the population.

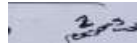
Fish can, of course, be overharvested. Many populations have been greatly reduced because so many individuals were harvested that too few reproductive adults survived to maintain the population. The Georges Bank off the coast of New England—a source of cod, halibut, and other prime food fishes—has been exploited so heavily that many fish stocks have been reduced to levels insufficient to support a commercial fishery.

The whaling industry has also engaged in excessive harvests. The blue whale, Earth's largest animal, was hunted nearly to extinction by the middle of the twentieth century. The industry then turned to smaller species of whales that were still numerous enough to support commercially viable whaling operations (Figure 54.14).

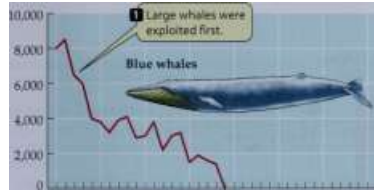
Management of whale populations is difficult for two reasons. First, unlike fish, whales reproduce at very low rates. They have long prereproductive periods, produce only one offspring at a time, and have long intervals between births. Thus many whales are needed to produce even a small number of offspring. Second, because whales are distributed widely throughout Earth's oceans, they are an international resource whose conservation and wise management depends upon cooperative action by all whaling nations. This continues to be difficult to achieve.

Life history information is used to control populations

The same principles apply if we wish to reduce the size of populations of undesirable species and keep them at low

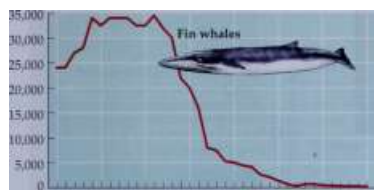


36,000



c. 4,000

1950 1955 1960 1965 1970 1975 1980 1985



1950 1955 1960 1965 1970 1975 1980 1985

POPULATION ECOLOGY 971

54.14 Overexploitation of Whales

The graphs show the numbers of whales of four species killed each year from 1950 to 1985. As each species reached low population levels, the whaling industry turned to other species. All four species were driven to very low levels by sustained hunting.

Similarly, if we wish to preserve a rare species, the most important step usually is to provide it with sufficient habitat. If habitat is available, the species will usually reproduce at rates sufficient to maintain its population. If the habitat is insufficient, preserving the species usually requires expensive and continuing intervention, such as providing extra food.

Humans have introduced many species to new habitats outside their native ranges. When these introduced species undergo population explosions, humans attempt to reduce their numbers by introducing new predators and parasites from the introduced species' original habitat. For example, the cactus *Opuntia*, introduced into Australia from

Progressively smaller whales were exploited more as populations of larger species decreased.



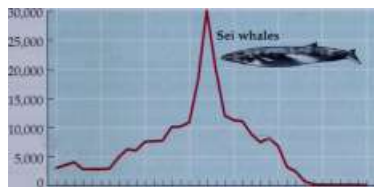
1950 1955 1960

1965 1970 Year

1975 1980 1985

South America, spread rapidly and became a common pest species over vast expanses of valuable sheep-grazing land. The cactus population was controlled by introducing a moth species (*Cactoblastis cactorum*) whose larvae feed on *Opuntia*. Once egg-laying females find a patch of cactus, their larvae completely destroy the patch (Figure 54.15).

But new patches of cactus arise in other places from seeds dispersed by birds. These new patches flourish until they are found and destroyed by *Cactoblastis*. Over a large region, the numbers of both *Opuntia* and *Cactoblastis* are today fairly constant and low, but in the local areas that make up the whole, there are periodic oscillations resulting



1950 1955 1960 1965 1970 1975 1980 1985

densities. At densities well below carrying capacity, populations have high birth rates and can therefore withstand higher death rates than they could closer to carrying capacity. Killing part of a population whose dynamics are influenced primarily by density-dependent factors only reduces it to the density at which it experiences the most rapid rate of growth. A far more effective approach to reducing the population of a species is to remove its resources, thereby lowering the carrying capacity of its environment. We can rid our dumps and cities of rats more easily by making garbage unavailable (reducing the carrying capacity of the rats' environment) than by poisoning rats.



54.15 Biological Control of an Introduced Pest

Cactoblastis caterpillars consume an Opuntia cactus in Australia.

972 CHAPTER FIFTY-FOUR

Old

Stone Vge

A

■ a 4

a. * o v a.

c 3 .n

c c

~



V>\v

Stone Age A

Bronze Iron

Middle Modern

Age

Age

Ages

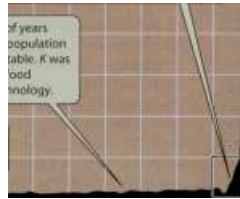
times

| Recently the human population has grown nearly exponentially. I cube twentieth century, the h uman population orewn nearly exponential ly.

| For thousands of years Earth's human population was relatively stable. K was set by existing food production technology.

I Agricultural revolution, the domestication of animals and plants, begins.

if



12,000 11,000 10,000 9000 8000 7000 6000 5000 4000 3000 2000 1000^Present

Years before present (BP)

54.76 Human Population Growth

Increases in Earth's carrying capacity for humans brought about by technology have allowed human populations to grow rapidly.

Q In October 1999, Earth's ---: population surpassed 6 billion people.



1000 800

600 400

200 Present

?'

from the extermination of first the plant and then the herbivore. This is another example of a series of subpopulations connected by occasional dispersing individuals.

Can we manage our own population?

Managing our own population has become a matter of great concern. For thousands of years, Earth's carrying capacity for human populations was set at a low level by food and water supplies and disease. Domestication of plants and animals and cultivation of the land enabled our ancestors to increase the resources at their disposal dramatically. These developments stimulated rapid population growth up to the next carrying capacity limit, which was determined by the agricultural productivity possible with only human- and animal-powered tools. Agricultural technology and artificial fertilizers, made possible by the tapping of fossil fuels, greatly increased agricultural production, further raising Earth's carrying capacity for humans. The development of modern medicine reduced the effectiveness of disease as a limiting factor on human populations, raising the global carrying capacity still further (Figure 54.16). Medicine and better hygiene have allowed people to live in large numbers in areas where diseases formerly kept numbers very low.

What is Earth's present carrying capacity for people? Today's carrying capacity is set by Earth's ability to absorb the by-products—especially CO₂,—of our enormous consumption of fossil fuel energy and by whether we are willing to cause the extinction of millions of other species to accommodate our increasing use of environmental resources. We will explore some of the consequences of high human population densities for the survival of other species in Chapter 58.

Chapter Summary

Population Structure: Patterns in Space and Time

- ▶ A population consists of all the individuals of a species within a given area.
- ▶ The number of individuals of a species per unit of area (or volume) is its population density.
- ▶ Individuals in a population may have uniform, random, or clumped distributions. Review Figure 54.2
- ▶ The age distribution of individuals in a population reveals much about the recent history of births and deaths in the population. The timing of births and deaths may influence age distributions for many years. Review Figure 54.3

Population Dynamics: Changes over Time

- ▶ Births, deaths, immigration, and emigration drive changes in population density and distribution.

- Life tables help us visualize patterns of births and deaths in a population. Review Table 54.1
- Graphs of survivorship in relation to age show when individuals survive well and when they do not. Review Figure 54.4

Patterns of Population Growth

- All populations have the potential to grow exponentially. However, no population can maintain exponential growth for very long because environmental limitations cause birth rates to drop and death rates to rise.
- The number of individuals of a particular species that an environment can support—called the carrying capacity—is determined by the availability of resources and by disease and predators.
- A population in a limited environment at first grows rapidly, but growth rates decrease as the carrying capacity is approached. Review Figure 54.5
- The overall densities of many populations are determined by "births" (colonizations) and "deaths" (extinctions) of local subpopulations. Immigrants may prevent declining subpopulations from becoming extinct, a process known as the rescue effect. Review Figure 54.6

Population Regulation

- Regulation of a population by changes in per capita birth or death rates in response to density is said to be density-dependent.
- If per capita birth and death rates are unrelated to a population's density, the population is not regulated.
- The density of a population is determined by the combined effects of all density-dependent and density-independent factors affecting it. Review Figures 54.7, 54.8

Life Histories Influence Population Growth

- The life history of a species describes how it divides its efforts among growth, dispersal, and reproduction over time.
- Trade-offs inevitably exist between number and size of offspring, between number of offspring and parental care, between survival and reproduction, and between growth and reproduction. Review Figure 54.12
- Reproductive value is the average number of offspring that remain to be born to individuals of a particular age. Reproductive value rises to a peak when individuals first begin to reproduce and declines to zero after reproduction ceases.
- Senescence—an increased probability of dying with increasing age—evolves because natural selection cannot act on alleles that first produce their phenotypic effects after a reproductive value drops to zero. Review Figure 54.13

Can Humans Manage Populations?

- Humans use the principles of population dynamics to control and manage populations of species they consider desirable or undesirable. Nevertheless, many populations have been overexploited. Review Figure 54.14
- Earth's carrying capacity for humans has been increased several times by technological developments. Review Figure 54.16

For Discussion

1. Huntington's disease is a severe disorder of the human nervous system that generally results in death. It is caused by a dominant allele that does not usually express itself phenotypically until its bearer is 35 to 40 years old. How fast is the gene causing Huntington's disease likely to be eliminated from the human population? Would your answer change if the gene expressed itself when its bearer was 20 years old? 10 years old?
2. Many people have improperly formed wisdom teeth and must spend considerable sums of money to have them removed. Assuming, as is probably the case, that the presence or absence of wisdom teeth and their mode of development are partly under genetic control, will we gradually lose our wisdom teeth by evolutionary processes?
3. Some organisms, such as oysters, cod, and elm trees, produce vast quantities of offspring, nearly all of which die before they reach adulthood. What fraction of such deaths are likely to be selective—that is, dependent on the genotypes of the individuals dying? What does your answer imply for the rates of evolution of oysters, cod, and elms?
4. In this chapter we identified a number of trade-offs in life history evolution. Why are these trade-offs inevitable? Why is knowledge about trade-offs important when we attempt to manipulate the life histories of organisms?
5. Ecologists often use the concept of carrying capacity when studying the growth and regulation of populations in nature, even though carrying capacity often changes markedly over time. How can the concept be useful if its value changes so often?
6. Most organisms whose populations we wish to manage for higher densities are long-lived and have low reproductive rates, whereas most organisms whose populations we attempt to reduce are short-lived but have high reproductive rates. What is the significance of this difference for management strategies and the effectiveness of management practices?

7. In the mid-nineteenth century, the human population of Ireland was largely dependent upon a single food crop, the potato. When a disease caused the potato crop to fail, the Irish population declined drastically for three reasons: (1) a large percentage of the population emigrated to the United States and other countries; (2) the average age of a woman at marriage increased from about 20 to about 30 years; and (3) many families starved to death rather than accept food from Britain. None of these social changes was planned at the national level, yet all contributed to adjusting population size to the new carrying capacity. Discuss the ecological strategies involved, using examples from other species. What would you have done had you been in charge of the national population policy for Ireland?

8. From a purely ecological standpoint, can the problem of world hunger ever be overcome by improved agriculture alone? What components must a hunger-control policy include?



Community Ecology

*4

Some people like their food spicy hot; others don't. The spices that impart strong flavors to foods are actually antioxidant, antimicrobial[^] and antiviral chemicals. These chemicals evolved because they protected the plants that produced them from predators and diseases. When we add spices during cooking, we borrow the plants' survival "recipes" and use them to protect our own food. The protection that spices provide was especially important before refrigeration and freezing were widely available, but even today, spices help prevent contamination of foods in most parts of the world.

Organisms interact with one another in a variety of ways. Some of these interactions involve eating and being eaten, but organisms may also interact competitively, or they may benefit one another. All organisms are potentially or actually food for some other organism, and most of them have evolved defenses that make them more difficult to find and capture, or less palatable or nutritious if they are captured. Consumers of those organisms have, in turn, evolved ways of getting around the defenses of their prey.

The organisms that live together in a particular area constitute a natural community. Each species interacts in unique ways with other species in its community and with its physical environment. Some of these interactions are strong and important; others are weak and affect the functioning of the community very little. The study of such interactions, and how they determine which and how many species live in a place, is the focus of community ecology.

For several decades, ecologists debated whether which species live together in communities is determined primarily by the interactions of individuals with the physical environment or by their interactions with other organisms. Some ecologists even suggested that a community is a superorganism in which each species plays a particular role, just as each organ plays a role within the body of an individual organism. That view has been abandoned because organisms, unlike organs, do not evolve under

Some Like It Hot

The peppers on the right of this photo are the seeds of a tropical vine, *Piper nigrum*. The hot chiles (upper left) are the fruits of an unrelated pepper plant, *Capsicum annuum*. The chemicals in peppers and other "hot" spices are often antimicrobial agents.

the influence of natural selection to serve their community. Nevertheless, determining the roles of species interactions with the biotic and abiotic environment is a major challenge for community ecologists today.

In this chapter we do consider interactions between organisms and their physical environment, but we concentrate on the major biological interactions and show how they influence the structure and functioning of ecological communities.



Types of Ecological Interactions

Organisms interact with one another in five major ways:

► Two organisms may mutually harm one another. This type of interaction is common when two organisms use the same resources and those resources are insufficient to supply their combined needs[^]. Such organisms are called competitors and their interactions constitute

competition ;

► One organism, by its activities, may benefit itself while harming another, as when individuals of one species eat individuals of another. The eater is called a predator or parasite, and the eaten is its prey or host. These interactions are known as predator-prey or parasite-host interactions.,



COMMUNITY ECOLOGY 975

\
C

- If both participants benefit from an interaction, we call them mutualists, and their interaction is a mutualism.
- If one participant benefits but the other is unaffected, the interaction is a commensalism.
- If one participant is harmed but the other is unaffected, the interaction is an amensalism.

These categories of species interactions are summarized in Table 55.1. But they are not clear-cut, both because the strengths of interactions vary and because many cases do not fit the categories neatly. Nevertheless, most interactions fit these categories well enough for us to use them as a guide for exploring interactions among species in this chapter.

Resources and Consumers

Many interactions between organisms within communities center on resources and their consumers. A resource is anything directly used by an organism that can potentially lead to the growth of the population and whose availability is

reduced when it is used. We usually think first of resources that can be consumed by being eaten, but space—including hiding places, nest sites, and establishment sites for sessile organisms—becomes unavailable if it is occupied, so it, too, is a resource. Factors such as temperature, humidity, salinity, and pH, even though they may strongly affect population size, are not resources because they can be neither consumed nor monopolized.

Some resources, such as nest sites, are not altered by being used and immediately become available for occupancy again when the user leaves. Other resources must regenerate before they are again available to consumers.

Biotic interactions influence the conditions under which species can persist

Each species can persist only under a certain set of environmental conditions, which define its ecological niche. If there were no competitors, predators, or disease organisms, in its environment, a species would be able to persist in a broader array of physical conditions (its fundamental niche) than it can in the presence of other species that negatively affect it (its realized niche). On the other hand, the presence of beneficial species may increase the range of physical conditions in which a species can persist.

An experiment performed on two species of barnacles, *Balanus balanoides* and *Chthamalus stellatus*, demonstrated the importance of both abiotic and biotic factors in determining the fundamental and realized niches of these two species. These barnacles live between high tide and low tide levels on rocky North Atlantic shores. Adult *Chthamalus* generally live higher in the intertidal zone than do adult *Balanus*, but young *Chthamalus* settle in large numbers in the *Balanus* zone.

In the absence of *Balanus*, young *Chthamalus* survive and grow well in the *Balanus* zone, but if *Balanus* are present,

they grow more poorly and die more rapidly than when they are alone.

Young *Balanus* settle in the *Chthamalus* zone, but they grow poorly because they lose water rapidly when exposed to air. *Chthamalus* compete successfully with them there, but *Balanus* would persist slightly higher in the intertidal zone in the absence of *Chthamalus*. By experimentally removing one or the other species, researchers have shown that the vertical ranges of adults of both species are greater in the absence of the other species. The result of their interaction is intertidal zonation, with *Chthamalus* growing above *Balanus* (Figure 55.1).

Limiting resources determine the outcomes of interactions

Resources whose supply is less than the demand made upon them by organisms are called limiting resources. Resources that are not limiting may have little influence on a species' population dynamics. For example, most terrestrial animals have strict but similar requirements for a certain minimum level of oxygen. However, studying the use of oxygen reveals very little about the structure of terrestrial communities because the concentration of oxygen, which is about 21 percent of the atmosphere, is nearly always above that minimum level.

The limiting resources that influence distributions and abundances of terrestrial species are those that are de-pletable and regenerate slowly, such as food. In freshwater aquatic environments, however, where the maximum concentration of dissolved oxygen is only 0.5 percent, organisms regularly deplete oxygen. Aquatic ecologists, unlike terrestrial ecologists, pay careful attention to oxygen levels.

Which resources are limiting differs among environments, but some kinds of resources, such as food supplies,

^s

976 CHAPTER FIFTY-FIVE

Balanus is distributed over a broad range of depths but is more sensitive to desiccation (drying out).

Chthamalus is more resistant to desiccation but is outcompeted by *Balanus* lower in the intertidal zone.

Spring high tide-Neap high tide

Mean

tidal

level

Neap low tide

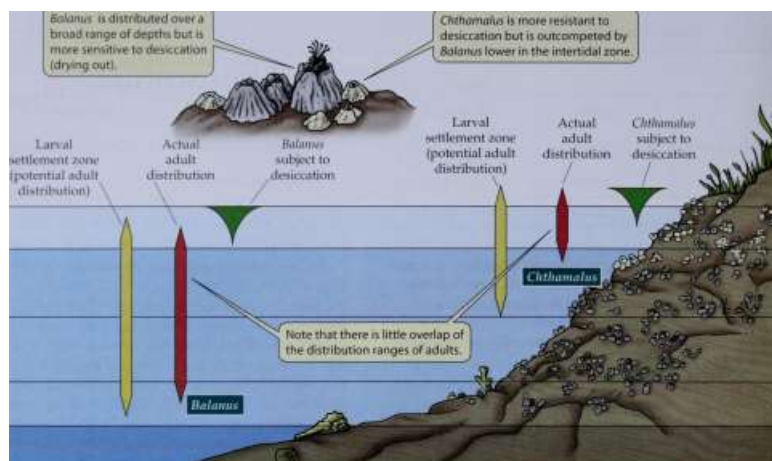
Spring low tide

Larval

settlement zone

(potential adult

distribution)



55.1 Potential and Actual Distributions of Two Barnacle Species

Integrating the relative importance of each resource makes the zone each species occupies smaller than the zone it could potentially occupy in the absence of the other species. The width of the red and gold bars is proportional to the density of the populations.

are often limiting. Because of the importance of resources in the lives of all species, we first examine competition that takes place among organisms needing scarce resources, and then consider predation.

Competition: Seeking and Using Scarce Resources

If two or more individuals use the same resources, and those resources are insufficient to meet their demands, the individuals are competitors, whether they are members of the same or a different species. Intraspecific competition — competition among individuals of the same species — may result in reduced growth and reproductive rates for some individuals, may exclude some individuals from better habitats, and may cause the deaths of others. Interspecific competition — competition among individuals of different species — affects individuals in the same way, but in addition, it can exclude one species; many are kept out of habitats where it can compete successfully, a phenomenon called competitive exclusion. In extreme cases, a competitor may cause the extinction of another species. In this section we will show how ecologists study competition and discuss how it influences species distributions and the composition of ecological communities.

Plants are good subjects for experiments to test the nature and results of competitive interactions because they compete for light, water, and nutrients, all of which can easily be manipulated. For example, the relative importance of root and shoot competition can be assessed by growing plants in shared or separate pots so that either the shoots or roots, or both, compete for resources: nutrients and water in the case of roots, light in the case of shoots. The results of an experiment

measuring root and shoot competition between a clover (*Trifolium repens*) and a grass (*Lolium perenne*) are shown in Figure 55.2. The grass outcompeted the clover in both root and shoot competition because a rich supply of nutrients, which the grass was able to use more efficiently than the clover, was provided.

Competition can restrict species' ranges

The role of competition in restricting the ranges of species is illustrated by the interaction of the two barnacle species described in Figure 55.1. In some cases, competition can completely exclude a species from part or all of its range.

Parasitic wasps were introduced into southern California to control outbreaks of scale insects that were seriously damaging citrus orchards. The Mediterranean wasp *Aphytis chrysomphali* was established in southern California by 1900, but it did not effectively control scale insects. Therefore, a close relative from China, *A. lingnanensis*, which has a higher reproductive rate, was introduced in 1948. *A. lingnanensis* increased rapidly, and within a decade it had displaced *A. chrysomphali* from most of its California range (Figure 55.3).

EXPERIMENT

Question: What are the relative effects of root and shoot competition on plant growth?

METHOD

No interspecific competition (control)



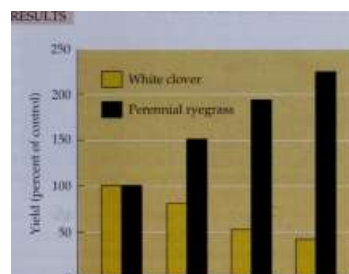
Perennial ryegrass White clover

Interspecific competition (experimental)



Root competition Shoot competition

Root and shoot competition



No inter-specific competition

Root competition

Shoot competition

Root and shoot competition

Conclusion: In this experiment, both root and shoot competition were important but shoot competition was more important.

55.2 Plants Compete with their Roots and Shoots

By growing plants in separate pots or together, experimenters can distinguish the influences of root and shoot competition on plant growth. An experiment using white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) showed that both roots and shoots of these plants are involved in competition.

Competition can reduce species' abundances

Often competition reduces the abundances of competing species rather than eliminating them from an area. Many species of seed-eating ants and rodents live together in the Sonoran Desert of Arizona. To determine whether competition between ants and rodents influences their abundances, ecologists removed ants from some sites, rodents from other sites, and both ants and rodents from a third set of sites. When they removed ants, the density of rodents increased slightly, but seed densities did not change. When they removed rodents, the density of ant colonies nearly doubled, but again, seed densities did not change. When they removed both ants and rodents, seed densities increased to five times their previous value. These results showed that ants and rodents were competing for and influencing their food supply, but that rodents had a much stronger effect on ants than vice versa, $v^{\wedge},^{\wedge}$ Jts. $\wedge s=\wedge=> C$ To determine whether different species of rodents also compete with one another, the ecologists erected rodent-proof fences around 50 x 50-m desert plots. The fences around the experimental plots had holes that small rodents could pass through, but were too small to allow the passage of large kangaroo rats. The holes in the fences surrounding control plots were large enough for all rodents to pass through. Within 2 years of the exclusion of kangaroo

Parasitic wasp

HHH A. chn/Hoiuphali ^] A. Ttngndnehsis



A. chrysomphali wasps were widely distributed in 1948 when *A. lingnanensis* was introduced.

Within 10 years, the new wasp had outcompeted the other species throughout most of its range.

55.3 A Species May Eliminate a Competitor from Parts of Its Range

Aphytis lingnanensis displaced *A. chrysomphali* over most of its range within a decade.

Y,

978 CHAPTER FIFTY-FIVE

rats from the experimental plots, densities of small seed-eating rodents increased more than twofold, and the plots without kangaroo rats supported more rodent species than the control plots. These results showed that kangaroo rats reduce populations of some rodent species and eliminate others from places where they live. Kangaroo rats compete with other seed-eating rodents both by reducing their food supply—exploitative competition—and by aggressively defending space—interference competition: I

In most natural communities, many species with similar ecological requirements live together, as seed-eating ants and rodents do in the Sonoran Desert. How can so many similar species share natural environments? Part of the answer is that natural environments are variable in space and time. That is why competing species often eliminate one another from some parts of the environment, but not from others. Also, natural environments typically provide many types of food, so that competing species do not overlap completely in their use of resources. In addition, other factors, such as predators, disease, and bad weather, may keep populations well below the environmental carrying capacity so that they rarely compete.

Predator-Prey and Parasite-Host Interactions

Competitive interactions in nature are often subtle, indirect, and difficult to detect. In contrast, predation is often direct, conspicuous, and easy to study. Consequently, our knowledge of predator-prey relationships is extensive. In this section we discuss how the dynamics of predator-prey and parasite-host interactions differ, and why interacting predator and prey populations typically fluctuate over time. Then we consider the evolutionary results of predator-prey interactions.

are typically larger than and live outside the

predators

bodies of their prey. Generally they kill prey individuals when they eat them. Parasites are smaller than their hosts and may live inside or outside their bodies. Parasites often live in or on their hosts without killing them and may live for many generations within a single host. As a result, parasite-host interactions differ in interesting ways from predator-prey interactions.

Parasite-host interactions

Some parasites are only slightly smaller than their hosts, but others, such as viruses and protozoa, which are called microparasites, are much smaller than their hosts. Microparasites are able to reproduce within their hosts because their generation times are much shorter than those of their hosts. A host may harbor thousands or millions of them.

To understand the dynamics of microparasite-host interactions, it is useful to divide a host population into three distinct classes: susceptible; infected; or recovered and immune. Changes in the numbers of individuals in each class depend on births, deaths, infections, and development and

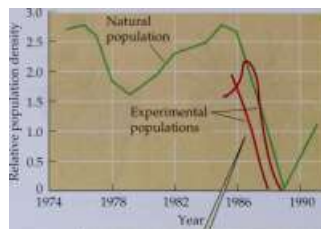
EXPERIMENT

Question: Do tent caterpillar populations crash due to food shortages or infection by a virus?

METHOD

RESULTS

Introduce virus-infected caterpillars into experimental caterpillar populations that have ample food.



The experimental populations crash at the same time as the natural population from which infected caterpillars were introduced.

N*

Conclusion: The virus, not food shortage, causes the caterpillar population to crash.

55.4 Microparasites Can Cause Population Crashes

Transferring infected tent caterpillars from a natural population that was about to crash to uninfected populations caused those populations to decline even though food was abundant.

loss of immunity. For a microparasite population to survive in a host population, on average at least one new host individual must become infected before each infected host dies.

A microparasite can readily invade a host population dominated by susceptible individuals, but as the infection spreads, fewer and fewer susceptible individuals remain. Eventually a point is reached at which infected individuals do not, on average, transmit the infection to at least one other individual. Then the infection dies out in the host population. As a result, microparasite infections typically rise, then fall, and do not rise again until a sufficiently dense population of susceptible host individuals has reappeared.

Interactions between a microparasite—a virus—and populations of its host—the western tent caterpillar (*Mala-cosoma californicum*)—have been investigated experimentally (Figure 55.4). Larval western tent caterpillars eat the leaves of a variety of species of deciduous trees and shrubs in North America. Their populations fluctuate dramatically, with peak densities occurring about every 10 years. Food supply cannot, by itself, drive these cycles, because populations often collapse before they defoliate the trees. Rather, a virus appears to be responsible. This virus typically kills an infected caterpillar within a few weeks. When it dies, the caterpillar ruptures and releases millions of viruses onto the leaves and bark of trees, infecting many other caterpillars. After an outbreak of the virus, tent caterpillar populations remain low for many years until the remaining viruses are killed by exposure to sunlight.

To test whether viruses drove the population cycles,

;

COMMUNITY ECOLOGY

r r

ecologists introduced caterpillars from infected populations into experimental sites where caterpillar populations had abundant food and were starting to increase. Caterpillars in the experimental populations became infected and were killed by the introduced virus; consequently, these experimental populations declined at the same time as the populations from

which the introduced caterpillars were drawn. In contrast, densities of caterpillars in control populations with abundant food continued to increase.



Predator-prey interactions

When a predator captures and eats a prey individual, it reduces the size of the prey population by one, but the effects of predators on prey population dynamics cannot be determined simply by counting the number of prey eaten. We also need to know how prey densities influence the ease with which prey are captured and how rapidly they reproduce. To understand the complex interactions between predators and their prey, it is useful to consider the process of predation from the perspective of an individual predator. Consider, for simplicity, a predator species that eats only one kind of prey. An individual predator can find enough to eat if the rate at which it encounters prey is above a certain threshold value. Below that threshold, it will lose weight and eventually starve. Nevertheless, the predator may continue to eat prey while slowly losing weight, driving



ing the prey population down further. Eventually the number of predators increased, which may allow the prey population to increase in numbers. This increase may, in turn, permit the prey population to increase further.

because of this pattern, fluctuation often lags behind the prey population. Predator-prey interactions often cause fluctuations in the densities of both populations, just as microparasites often cause fluctuations in populations of their hosts.

Population density changes among small mammals and their predators living at high latitudes are the best-known examples of predator-prey oscillations. Populations of Canadian lynx and their principal prey, hares, oscillate on a 9- to 11-year cycle. For many years, these numbers were thought to be driven only by interactions between hares and lynxes. Recently, however

ecologists asked whether any part of the lynx-hare oscillation could be explained by fluctuations in food supply in addition to predation.

To answer this question, the ecologists set up 1-km² blocks of undisturbed coniferous forest in Yukon Territory, Canada. In two of the blocks, the hares were given supplemental food year-round. An electric fence with a mesh large enough to allow hares, but not lynxes, to pass through was erected around two of the blocks. In one of these blocks, extra food was provided. In two other blocks, fertilizer was added to the quality of plant food for the hares. The two blocks served as unmanipulated controls.

These experiments produced striking results. Excluding lynxes doubled, and adding food tripled, hare densities. The increase and decline phases of a cycle. Predator exclusion combined with food addition



&

hare density" 11-to 12%:

no effect on hare population density

55.6). Thus, the ecologists concluded that

cycles are driven both by predation and by

by interactions between hares and lynxes:

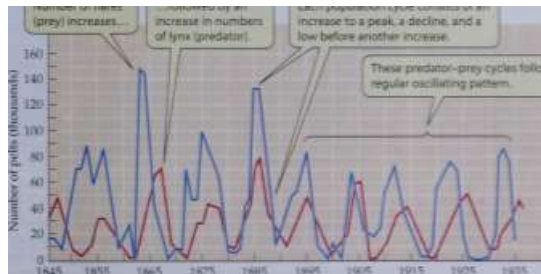
supply.

Number of hares (prey) increases...

...followed by an increase in numbers of lynx (predator).

Each population cycle consists of an increase to a peak, a decline, and a low before another increase

These predator-prey cycles form a regular oscillating pattern.



J

185:

:

Predators may eliminate prey from some environments but

not others

In heterogeneous environments, predators limit their prey

in some ponds on islands of Lake Superior,

55 Hare and Lynx Population Cycle in Nature

: - 1 year: : : mw-

show the rate and the "Canadian lynx" number of pelts sold by fur trappers'

• - - : : : :

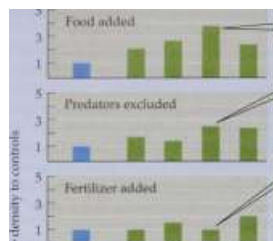
980 CHAPTER FIFTY-FIVE

EXPERIMENT

Question: Do food supply, food quality, and/or predation influence population cycles of hares?

METHOD Food, predators, and fertilizer are manipulated singly or in combination while hare populations are monitored.

RESULTS



Adding food tripled the hare density.

Excluding predators doubled hare density.

Fertilizing vegetation to increase its food quality had no significant effect.

2 17

| 15

& 13 11 9 7 5 3 1

Food added and predators excluded



Adding food and excluding predators increased hare density dramatically.

—

Control

Phase of hare population cycles

Conclusion: Both food supply and predation affect hare population cycles.

55.6 Prey Population Cycles May Have Multiple Causes

Experiments showed that both food supply and predation (but not food quality) affect the population densities of snowshoe hares.

chorus frogs (*Pseudacris triseriata*) are found in only some of the habitats that seem suitable for them. Three major predators—the larvae of a salamander, nymphs of a large dragonfly, and dytiscid beetles—eat chorus frog tadpoles. An ecologist noticed that the tadpoles were common in ponds containing beetles, but were rare in ponds with salamander larvae and dragonfly nymphs. In laboratory experiments, he established that the salamander larvae could eat only small tadpoles, but that dragonfly nymphs could eat tadpoles of all sizes. Therefore, he hypothesized that dragonfly nymphs were responsible for eliminating chorus frogs from many ponds.

To test this hypothesis, he selected two large ponds that contained dragonfly nymphs but no tadpoles, and two other ponds that contained tadpoles but no nymphs. So that all the tadpoles would be handled equally, he removed the tadpoles from the two ponds that lacked dragonfly nymphs and then reintroduced them at the same densities. He introduced dragonfly nymphs into one of those ponds at typical densities. He also removed nearly all dragonfly nymphs from one of the ponds that had them and then introduced tadpoles into both of those ponds.

The dramatic results of the experiment supported his hypothesis (Figure 55.7). Tadpoles were eliminated from ponds with dragonfly nymphs, but survived well in ponds from which dragonfly nymphs were absent, or nearly so. This experiment shows that a particular predator may eliminate its prey in certain environments; however, it does not tell us why dragonfly nymphs were naturally absent from some of the ponds.

Predator-prey interactions change over evolutionary time

Because predators do not capture prey individuals randomly, they are agents of natural selection as well as agents of mortality. As a consequence, prey species have evolved a rich variety of adaptations that make them more difficult to capture, subdue, and eat. Among the evolutionary adaptations of prey are toxic hairs and bristles, tough spines, noxious chemicals and the means for ejecting them (Figure 55.8), camouflage, and mimicry of inedible objects or of larger or more dangerous organisms. Predators, in turn, may evolve to be more effective at overcoming prey defenses, leading to an evolutionary "arms race."

mimicry is an evolved defense. Among the best-studied adaptations of prey to predation is mimicry, an evolved resemblance to, some inedible or unpalatable item. In Batesian mimicry, a palatable species mimics an unpalatable or noxious one. Examples are the mimicry of ants by spiders and of bees and wasps by many different insects (Figure 55.9). Batesian mimicry works because a predator that captures an individual of an unpalatable or noxious species learns to avoid any prey of similar appearance.

However, if a predator captures a palatable mimic, it is rewarded with food, and it learns to associate palatability with the appearance of that prey. As a result, individuals of unpalatable species are attacked more often than they would be if they had no Batesian mimics. Because unpalatable individuals that differ from their mimics more than the average are less likely to be attacked by predators that have eaten a mimic, directional selection causes unpalatable species to evolve away from their mimics. Batesian mimicry systems are stable only if a mimic evolves toward the appearance of an unpalatable species faster than the unpalatable species evolves away from it, which usually requires that the mimic be less common than the unpalatable species.

Another type of mimicry is Mullerian mimicry, the convergence over evolutionary time in the appearance of two

EXPERIMENT

Question: Do dragonfly nymphs eliminate chorus frog tadpoles from otherwise suitable ponds?

METHOD Predators and prey are added to ponds in different combinations RESULTS

Experiment 1 Ponds 1 and 2 had no dragonfly nymphs and many tadpoles.

Pond 1 had no dragonfly nymphs; many tadpoles, and no nymphs were introduced.

300 -

« 250

.§•200

£ 150 6 100 -50 0

14 21

Days

Pond 2 had no dragonfly nymphs; many tadpoles, and 33 nymphs were introduced.

300

250 200

150

100

50

0

JZL

(o)

7 14 21 Days

Experiment 2 Ponds 3 and 4 had many dragonfly nymphs and no tadpoles.

In pond 3, all but 7 dragonfly nymphs were removed, and 112 tadpoles were added.

In pond 4, 95 dragonfly nymphs were present, and 234 tadpoles were added.

300

J 25 ° | -200

2 1 50 ~

6 100 -

2 50

0

6 12 22 Days

300 250 200 | _

150

100

50

0

(o) (o) (o)

6 12 Days

22

Conclusion: Dragonfly nymphs eliminate tadpoles from suitable ponds.

55.7 Predators Exclude Prey from Some Habitats

The speed with which dragonfly nymphs can eliminate tadpoles of the chorus frog from a pond is illustrated by the results of experiments in which dragonfly nymphs were added to ponds containing tadpoles.



(a) *Brachinus* sp.

(b) *Pterois volitans*

55.8 Defenses of Animal Prey

(a) A bombardier beetle ejects a noxious spray at the temperature of boiling water in the direction of a predator. The spray is ejected in high-speed pulses more than 20 times in succession, (b) The Indo-Pacific lionfish is among the most toxic of all reef fishes. Glands at the base of its spines can inject poison into an attacker. Its bright markings are thought to warn potential predators of this capability.

982 CHAPTER FIFTY-FIVE



55.9 A Batesian Mimic Falsely Advertises Danger

By mimicking a wasp, this ctenucid moth is protected from predators.

or more unpalatable species. All species in a Müllerian mimicry system, including the predators, benefit when inexperienced predators eat individuals of any of the species because the predators learn rapidly that all species of similar appearance are unpalatable. Some of the most spectacular tropical butterflies are members of Müllerian mimicry systems (Figure 55.10), as are many kinds of bees and wasps.

EVOLVED CHEMICAL DEFENSES ARE WIDESPREAD among plants. In addition to physical defenses against herbivores, such as tough leaves, hairs, or spines, most plant tissues also contain defensive chemicals called secondary compounds. There are two types of defensive secondary compounds: acute toxins and digestibility-reducing compounds.

[Acute toxins disrupt herbivore metabolism. Some of these toxins, such as nicotine, interfere with the transmission of nerve impulses to muscles. Others are hallucinogens, which cause individuals that ingest them to have a seriously distorted view of their environment. Some toxins imitate insect hormones and prevent insects from completing metamorphosis. Still others are unusual amino acids that become incorporated into herbivore proteins and interfere with their functioning. Other acute toxins defend

plants against viruses and bacteria. As we saw at the beginning of this chapter, most of the spices used in human cuisines have antibiotic properties. We can safely include them in our food because they are toxic only to microorganisms.

Digestibility-reducing compounds are secondary compounds that make plant tissues difficult to digest. The most common of these substances are tannins, which are present in the leaves of some herbaceous and most woody species. When an herbivore chews on a leaf, tannins are released from the intracellular compartments in which they are stored. They bind to proteins in the leaf and to the herbivore's digestive enzymes, reducing the ability of the herbivore to extract proteins from the leaves. Tannins may be present in such large quantities that watery drainage from forests dominated by tanniferous plants are tea-colored.

Neutral and Beneficial Interspecific Interactions

During predator-prey and competitive interactions, one or both participants in the interaction are harmed \ Amensal-



1 Highly unpalatable or noxious

J Moderately unpalatable or noxious

I Highly palatable (Batesian mimics)

□ Palatability not yet tested with birds

* Müllerian mimics of

butterflies in the same group

55.10 Müllerian and Batesian Mimics

By converging in appearance, the unpalatable Müllerian mimics among these different species of Costa Rican butterflies and moths reinforce each other in deterring predators. The palatable Batesian mimics benefit because predators have learned to associate these color patterns with distasteful or noxious effects.



55.17 Danger Comes From Above

Shrubs and herbaceous plants are often damaged by branches falling from tall trees.

IsrQ cause s harm to one of the partners without affecting Jhejither,. In the other two types of interspecific interactions—commensalism and mutualism—neither partner is harmed, and one or both may benefit. We examine these interactions in the sections that follow.

In a men s alism and commensalism, one participant is unaffected

\Amen salisms^i n which an individual harms another organism but is unaffected by the species it harms, are widespread and important in nature. Ma mmals, for example, create bare spaces aroun d water holes. They benefiL by dnnking~vvat er, but not by tramp ling t he plants they kill. Leaves and branches falling from trees damage smaller plants beneath them (Figure 55.11). The trees drop these old structures regardless of whether or not they damage other plants.

\ ^Commens alism\)enefits one partner but has no effect on the other. An example is the relationship between cattle egrets and grazing mamm als. Cattle egrets are found throughout the tropics and subtropics. They typically forage on the ground around cattle or other large mammals, concentrating thpirj^tigntin n npar thp hpads and feet of the m ammals, where they catch insects fl nshpH by thpir hooves an d m ouths (Figure 55.12). Cattle egrets that forage close to grazing mammals capturemo re food for le ss effort than egre ts that d o not. The benefit t o the egrets is clear ; the mammals neith er gair fnor lose!

Mutualisms benefit both participants

VMutualism^ are interactions that benefit both participants. Mutualistic interactions ex ist between plants a nd microorganisms, protists a nd fungi, plants and insects, _a nd among jglants. Animals also have mutualistic interactions with pro-

tists and with one another. As you learned in Chapter 27, the ev olution of eu karyotic organisms is believed to be the .^result of mutualistic interactions between previously free-living prok~aryotes and the cells they originally infected.

intergroup mutualisms. Some of the most complex and ecologically important mutualisms are between members of different kingdoms or domains.

Most plants have beneficial associations with soil-inhabit ing fun gi called mycorrhizae that enhance the~plaht's abil-ity to extract mine rals from the soil (see Figure 30.16). And in the critical mutualistic relationship of some plants with nitrogen-fixing bacteria of the genus Rliizobium (discussed at length in Chapter 36), the bacteria receive protection and nutrients from their host plant while providing the host with usable nitrogen.

Lichens are compound organisms consisting of highly mod ified fungi t hat harbo r cyanobacter ia)r_ green algae among their hyphae (see Figure 30.18). The fungi absorb water and nutrients from the environment and provide these as well as a supporting structure for the mlcroorgan-isms, w hich in turn provide the fungi with the pr oducts of photosynthesis.

"Animals have important mutualistic interactions with protists. For example, corals, some anemones, and some tunicates gain most of their energy from photosynthetic protists that live within their tissues. In exchange, they provide the protists with nutrients from the small animals they capture (see Figure 4.16c).

Termites have nitrogen-fixing protists in their guts that help them digest the cellulose in the wood they eat. Young termites must acquire their protists by eating the feces of other termites. If prevented from doing so, they soon die. The protists are provided with a suitable environment in which to live and an abundant supply of cellulose.



55.12 Commensalism Benefits One Partner

Cattle egrets catch more insects with less effort when they forage around large grazing mammals, such as cape buffalos. The buffalos are neither harmed nor helped by the egrets.

984 CHAPTER FIFTY-FIVE



55.73 A Plant-Animal Mutualism

Some Acacia species have large, swollen, hollow thorns in which ants build their nests.

thorns in which ants of the genus *Pseudomyrmex* construct their nests and raise their young (Figure 55.13). These ants live only on acacias. They feed on nectar that the trees produce at the bases of their leaf petioles and on special nutritive bodies on the leaves. The ants attack and drive off leaf-eating insects, eat the eggs and larvae of herbivorous insects, and sting browsing mammals. They also block the tips of other plants that grow over their host tree. The ants get room and board; the

plants get protection against herbivores and competing plants.

Experiments in which acacias are deprived of their ants demonstrate the amount of protection the ants provide (Figure 55.14).

Many angiosperms depend on animals to transport both their pollen and the seeds. The plants benefit from pollination by having their pollen carried to other conspecific plants and by receiving pollen to fertilize their ovules. Animals benefit by obtaining food in the form of nectar and pollen (Figure 55.15a). Plants also benefit from having their seeds dispersed to sites where they are more likely to germinate and survive than directly under the parent plant (Figure 55.15b). Animal dispersers benefit by eating the nutritious fruits surrounding the seeds. The plants pay a price for the benefits they receive: The energy and materials a plant uses to produce nectar, fruits, and other rewards for animals cannot help with or seed production.

■ c^

y&>

-r^

ANIMAL-ANIMAL MUTUALISMS. Many

species of ants have mutualistic relationships with aphids. Ants "milk" the small, plant-sucking insects by stroking the m

with their forelegs and antennae. The aphids respond by secreting droplets of partly digested plant sap that has passed through their guts. In return, the ants protect the aphids from predatory wasps, beetles, and other natural enemies. The aphids lose nothing, because plant sap is high in sugar but low in amino acids. Thus, the aphids inevitably inject more sugar than they can use.

plant-animal mutualisms. Terrestrial plants have many mutualistic interactions with animals. A complex mutualism between trees and ants that live in Central America illustrates the benefits of such interactions. Trees of the species *Acacia cornigera* have large, hollow

55.14 An Experiment Demonstrates the Benefits of Housing Ants

Acacia trees that housed ant colonies grew back faster than acacias without ants.

EXPERIMENT

Question: Do ants provide effective protection for acacia trees?

METHOD Acacia trees were severely pruned. Ants were allowed to recolonize some trees as they regrew, but not others.

RESULTS With ants

Without ants



Trees grown without ants were heavily attacked by other insects and regained their leaves very slowly.

Conclusion: Ants provide very effective protection for acacia trees.



(a) *Glossophaea* sp.



(b) *Boscophila garrulus*

55.15 Plants Incur Costs to Attract Mutualists

(a) Some animal pollinators such as this long-tongued bat are attracted by rewards of nectar or pollen, (b) The nutritious pulp of fruits are attractive to many birds, such as this Bohemian waxwing.

COMMUNITY ECOLOGY 985

influenced by interactions with a wide variety of predators, parasites, prey, or mutualists. For example, most flowers are pollinated by a number of animal species and most

pollinators visit many species of flowers.

The traits of fruits that surround many seeds are also the result of diffuse coevolution. Few fruits are adapted for dispersal by only a few species of animals. Most bird-dispersed fruits are red, or some combination of red and another color, and have no obvious odor to humans. Fruits dispersed by nocturnal animals, many of which lack color vision, are typically "purple," are not highly visible to birds, and usually have a pleasant odor. Bat-dispersed fruits are typically green and have a fruity odor. They are inconspicuous during the day, but are easy for bats to detect at night. Many bird-dispersed fruits have unpleasant tastes to mammals.

Species-specific coevolution is much rarer than diffuse coevolution, but yucca plants and the moths of the genus *Tegeticula* that pollinate them have this kind of relationship. Female yucca moths lay their eggs only in the ovules of yucca flowers, and yucca flowers are pollinated only by *Tegeticula*. A female *Tegeticula* lays no more than five eggs in any one flower. After she has laid her eggs, she scrapes pollen from the flower's anthers, rolls it into a small ball, flies to another yucca plant, and places the pollen ball on the stigma of a flower before laying another batch of eggs. When the eggs hatch, the larvae burrow into the ovary and feed upon the developing seeds. Each yucca species has a specific moth species associated with it (Figure 55.17).

One feature of the coevolved relationship between *Tegeticula* and *Yucca* is surprising: Why do female moths lay so few eggs per flower? Wouldn't a female moth that laid more than five eggs per flower produce more surviving off-

Interactions between plants and their pollinators and seed dispersers are clearly mutualistic, but they are not purely mutualistic. Many seed dispersers are also insect predators that destroy some of the seeds they remove from plants. Some organisms that collect these rewards are not mutualists at all. Many animals visit flowers without transferring any pollen. Some of them cut holes to get to the nectar-producing regions at the base of the flowers. On the other hand, some plants exploit their pollinators. The flowers of certain orchids, for example, mimic female insects, enticing male insects to copulate with them (Figure 55.16). The male insects neither sire any offspring nor obtain any reward, but they transfer pollen between flowers, benefiting the orchid.

Coevolution of Interacting Species

The relationships between plants and their pollinators and seed dispersers show how the evolution of species traits can be influenced by interactions with other species. Species that have mutually influenced one another's evolution are said to have



many species' traits are

55.16 Some Orchids Mimic Female Insects

The flowers of this orchid so closely resembles a female wasp that male wasps are tricked into attempting to copulate with

them, as this male is doing. The orchid gets pollinated, but the wasp gets no reward.

986 CHAPTER FIFTY-FIVE



55.17 Yucca- Yucca Moth Coevolution

The Joshua tree (a) is pollinated only by the yucca moth (b), shown here on a flower. The moth's larvae feed on developing yucca seeds fertilized by the pollen she transports.



{a) *Yucca brevifolia*

(b) *Tegeticula yuccasella*

spring than moths that lay only the usual number? The evolutionary reason for their restraint is that *Yucca* plants abort flowers in which more than five eggs are laid. As a result, no moth offspring are produced in such flowers. Thus, the mutualism is stabilized at a level that represents an "evolutionary compromise" between the fitness of the moths and the yuccas.

Some Species Have Major Influences on Community Composition

Organisms influence the communities in which they live through all of the types of interactions that we have just described. Through these interactions, they may influence the species richness of their communities—that is, the number of species that live there. They also influence their communities by altering microclimate, soil structure and chemistry, and water movement. These alterations affect the suitability of the physical environment for other organisms. Organisms also change the amounts and distributions of resources in the community. Species whose influences on ecological communities are greater than would be expected on the basis of their abundance are called keystone species. Keystone species may influence the species richness of communities, the flow of energy and materials in ecosystems, or both.

Some species have major influences on their communities simply through their abundance. For example, in terrestrial communities, plants form most of the structural environment, are the major modifiers of the physical environment, and are the pathway through which energy and nutrients enter communities. Anyone who has walked into the shade of a tree on a hot, sunny day knows that climate near the ground is strongly influenced by plants.

Animals may change vegetation structure and species richness

Animals that are able to change vegetation structure alter the environment for many other species, for example, beavers, to create meadows by cutting down trees and create ponds by building dams. Large grazing and browsing mammals may also dramatically change the structure and composition of vegetation.

To determine the influence of bison (*Bison bison*) on prairie vegetation, 30 individuals were introduced to the Konza Prairie Research Natural Area in northeastern Kansas, the largest tract of unplowed tallgrass prairie in North America. The herd, now about 200 animals in size, has unrestricted access to 10 watersheds that are regularly burned in spring. Bison prefer to graze on and eat few of the forbs (flowering plants) that grow among the grasses. Bison also prefer to graze on recently burned areas.

Areas from which bison are excluded and which are burned annually are dominated by tall grasses and have few plant species. In contrast, regularly burned areas that are grazed by bison have more forbs—and many more species of forbs—because the bison, by preferentially grazing on the grasses, create space for them (Figure 55.18). Also, urea in bison urine is hydrolyzed to ammonium and its nitrogen is available to plants within a few days; decomposing plant litter releases nitrogen

much more slowly. Therefore, plants in areas grazed by bison have higher leaf nitrogen levels and grow faster.

Predators may change marine community structure

Predation by the sea star *Pisaster ochraceus*, an abundant animal in rocky intertidal communities on the Pacific coast of North America, increases local species richness. In the

Biosphere

55.78 Bison Increase Plant Species Richness and Productivity

By grazing preferentially on grasses, bison increase the density of forbs and overall plant productivity.

600-500

2400

g IS

TJC 3 Os-bD

01

> 0

<

300

200

100

Forbs

n

After grazing by bison there are fewer grasses...

Grasses

.but more forbs.

Ungrazed

H

Grazed

.230

15

1 ^

£ 20-0

X.

a,

1310

a;

£ 5

-2 "3 « n

Grasses fertilized by bison urine photosynthesize faster.

, Grazed

Ungrazed

Llu I

absence of sea stars, their preferred prey, the mussel *Mytilus californianus*, crowds out other competitors in a broad belt of the intertidal zone. By consuming mussels, *Pisaster* creates bare spaces that are taken over by a variety of other species (Figure 55.19).

The influence of *Pisaster* on community composition was demonstrated by experimentally removing them from selected areas in the intertidal zone repeatedly over a 5-year period. These removals resulted in two major changes. First, the lower edge of the mussel bed extended farther down into the intertidal zone, showing that sea stars are able to eliminate mussels completely where they are covered with water most of the time. Second, and more dramatically, 28 species of animals and algae disappeared from the removal zones, until only *Mytilus*, the dominant competitor, occupied the entire substrate. By altering competitive relationships, predation by *Pisaster* largely determines which species live in these rocky intertidal communities.



Temporal Changes in Communities

Because organisms alter soil structure and chemistry and microclimates, the species composition of ecological communities changes constantly over time. The plants that first colonize a site after a disturbance, for example, differ from those that dominate the site later. Such a sequence of change in the species composition of a community is called ecological succession. Patterns and causes of ecological succession are varied, but the early colonists often alter the environment for arriving species grow.

Ecologists divide succession into two major types. Primary succession begins with the establishment of organisms on newly available sites that previously had no organisms. Secondary succession begins when organisms reestablish themselves on disturbed sites where some organisms survived the disturbance.

55.19 Sea Stars are Keystone Predators

This sea star (*Pisaster ochraceus*) is resting on a bed of mussels (*Mytilus* sp.), its preferred prey.

A good example of primary succession is the sequence of changes following the retreat of a glacier in Glacier Bay, Alaska, over the last 200 years. The retreating glacier left a series of moraines —gravel deposits formed where the glacial front was stationary for a number of years. No scientist was present to measure changes over the 200-year period, but ecologists have inferred the temporal pattern of succession by examining plant communities on moraines of different ages. The youngest moraines, close to the current glacial front, are populated with bacteria, fungi, and photo-synthetic microorganisms. Slightly older moraines have lichens, mosses, and a few species of shallow-rooted herbs. Successively older moraines have shrubby willows, alders, and conifers.

By comparing moraines of different ages, ecologists deduced the pattern of plant succession and of changes in the soil at Glacier Bay. Succession was caused in part by changes in the soil brought about by the organisms themselves. An herbaceous plant, *Dryas*, and alder trees have nitrogen-fixing bacteria in nodules on their roots. Because nitrogen is virtually absent from glacial moraines, nitrogen fixation by *Dryas* and alders improved the soil for the

988 CHAPTER FIFTY-FIVE

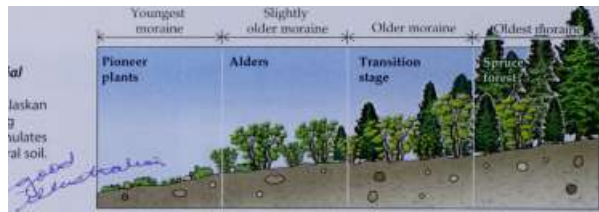
55.20 Primary Succession on a Glacial Moraine

As the plant community occupying an Alaskan glacial moraine changes from pioneering plants to a spruce forest, nitrogen accumulates both on the forest floor and in the mineral soil.

Youngest moraine

Slightly older moraine

Older moraine ^ ^ Oldest moraine



growth of spruce trees. Spruces then displaced the ones not

outcompeted and

at a dersi flheTTocal climate

300

250

C 3 (J oj

200

150 -

c n

to oi O (X

£ to 100 2 w

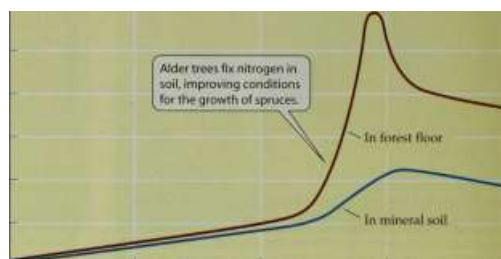
50

change dramatically, a forest community dominated by spruces is likely to persist for many centuries at Glacier Bay (Figure 55.20).

The changes that take place when all or part of the dead body of some plant or animal is decomposed are examples of/secondary succession] The succession of fungal species that decompose pine needles in litter beneath Scots pines (*Pinus sylvestris*) is shown in Figure 55.21. New litter is continually

ously deposited under pines, so that the surface layer of litter is young and deeper layers are progressively older. Decomposition begins when the first group of fungi starts consuming the needles as soon as they fall. Each group of fungi decomposes certain compounds, converting them to other compounds that are decomposed by the next successional group. This process continues over about 7 years, by which time the last group of organisms—basictiorrtycetes— has decomposed the litter completely.

Alder trees fix nitrogen in soil, improving conditions for the growth of spruces.



In mineral soil

50

100 Year

150

200

Indirect Effects of Interactions among Species

In the experiments we have described above, one member of a community was removed or added, and investigators measured the resulting changes. Single-species removals or additions can demonstrate the direct effects of species on one another, but to quantify indirect effects, observations and manipulations of several species are needed.

Q Freshly fallen needles decompose slowly over a 7-year period.

Coniosporium

t

Fungi of several genera aid in successive decomposition of needles.

Living needles o Freshly fallen needles

Slightly

decomposed

needles

Moderately

decomposed

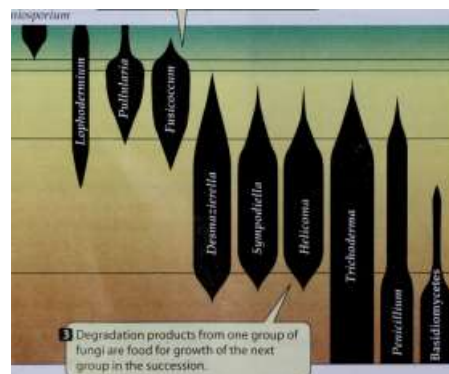
needles

Highly

decomposed

needles

7 years



55.21 Secondary Succession on Pine Needles

The abundances often types of fungi (indicated by the widths of the black bars) in pine needle litter change with time. The age of the needles increases with depth within the layer.



II Degradation products from one group of fungi are food for growth of the next group in the succession.

(a)

O Healthy oaks produce more acorns.

§J Dense mouse populations keep gypsy moth populations low by eating gypsy moth pupae.

7h^ Q Oak trees produce

large crops of acorns every few years.



f

Mouse populations increase greatly in years of heavy acorn production.

55.22 Direct and Indirect Interactions Control Populations

Several species, including mice, gypsy moths, and oak trees, interact to influence one another's population dynamics, (a) This is the pattern when mouse populations are dense, (b) When predators seriously depress mouse populations, this dynamic occurs.

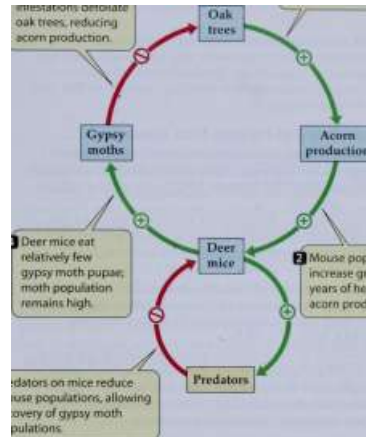
(M

o Gypsy moth

infestations defoliate oak trees, reducing acorn production.

^ ^ ^ El Oak trees produce

large crops of acorns I every few years.



o Deer mice eat relatively few gypsy moth pupae; moth population remains high.

Mouse populations increase greatly in years of heavy acorn production.

Predators on mice reduce mouse populations, allowing recovery of gypsy moth populations.

e*

Ecologists have assembled a variety of data to help them understand relationships between oak forests and the animals that eat their leaves and acorns. Most damage to leaves in the oak forests of eastern North America is caused by gypsy moths; most acorns are eaten by mice, chipmunks, deer, squirrels, and birds (Figure 55.21). Ineabundance of mice and chipmunks is controlled largely by _acorn abundance, which varies greatly from year to year. Deer consume large quantities of acorns during years of high acorn production, but during poor acorn years they shift to other foods.

Gypsy moths eat oak leaves. During years when their populations are very large, they may defoliate large expanses of forest. Outbreaks of gypsy moths occur once every 6-10 years. Gypsy moth populations collapse after they defoliate a forest because most larvae die of starvation. In the year following defoliation, oak trees are full of leaves, but gypsy moth populations remain low for many years. Why don't they quickly rebound?

To determine whether predation by mice prevented gypsy moth populations from recovering after a crash, ecologists measured predation rates by attaching freeze-dried gypsy moth pupae to small squares of burlap. They placed the burlap panels on oak tree trunks at sites where gypsy moths typically pupate. During a year of moderately dense mouse populations, all of the pupae were eaten within 8 days. During a year of low mouse density, half of the pupae remained after 18 days, which is longer than it would take them to complete metamorphosis and emerge. These results suggest that in years when acorns are abundant, mice keep gypsy moth populations at low densities by eating

most of their pupae. In so doing, they allow the oak trees to recover from defoliation and accumulate enough energy reserves to produce another large crop of acorns. Predators typically reduce mouse populations within 1.5 years after a year of high acorn production, after which gypsy moth populations rebound and again defoliate the trees. If few mice were present, the gypsy moths might rebound so quickly that the oaks could never produce large crops of acorns. If the investigators had studied only the interactions between mice and acorns or between gypsy moths and oak trees, they would not have discovered the important influences of other species on these interactions.

Chapter Summary

Types of Ecological Interactions

► Species interact with one another in five major ways: competition, predator-prey or parasite-host interactions, mutualism, commensalism, and amensalism. Review Table 55.1

Resources and Consumers

► A resource is anything directly used by an organism that can potentially lead to the growth of the population and whose availability is reduced when it is used.

► Biotic interactions, as well as the physical environment, influence the conditions under which species can persist. Review Figure 55.1

Competition: Seeking and Using Scarce Resources

► If organisms use the same resources and those resources are in short supply, the individuals are competitors. Competition may be either intraspecific or interspecific.

990 CHAPTER FIFTY-FIVE

► Plants are good subjects for competition studies because the resources for which they compete are easily manipulated. Review Figure 55.2

► Competition may restrict species ranges. Review Figure 55.3

► Species that use similar resources commonly coexist in nature because nature is spatially and temporally complex, many resources typically are available, and other factors often keep populations below carrying capacity so that they do not compete strongly.

Predator-Prey and Parasite-Host Interactions

► The relative sizes of predators and prey influence their interactions. Microparasites are typically much smaller than their prey and may live in or on their hosts without killing them.

► To understand the dynamics of host-microparasite interactions, it is useful to divide a host population into three distinct classes—susceptible, infected, and recovered and immune.

► Experimental manipulation of parasites and predators in nature reveals that they are often important in determining both numbers and distributions of their prey. Review Figures 55.4, 55.5, 55.6, 55.7

► Predators act as evolutionary agents by selecting for adaptations to protect against them. Prey have evolved many such adaptations, such as toxic hairs and bristles, tough spines, noxious chemicals, and mimicry of inedible objects or dangerous organisms. Review Figure 55.10

Neutral and Beneficial Interspecific Interactions

► Commensal interactions, in which one partner benefits while the other is unaffected, are common in nature.

► Mutualistic interactions, in which both participants benefit, are also common in nature. Mutualistic interactions occur between members of different groups of organisms (between plants and prokaryotes, between fungi and protists, and between animals and protists). Animals have mutualistic interactions with other animals and with plants, such as pollination and seed dispersal. Review Figure 55.14

Coevolution of Interacting Species

► Some mutualistic relationships, such as those between yuccas and yucca moths, are tightly coevolved, but diffuse coevolution between many species is much more common.

Some Species Have Major Influences on Community Composition

► Keystone species have influences on ecological communities that are greater than would be expected from their abundances, but abundant species also have major influences on community structure.

^ Vascular plants, mammals that change vegetation structure, predators on dominant competitors, and microorganisms often have major influences on ecological communities. Review Figure 55.18

Temporal Changes in Communities

► Ecological succession involves changes in the species composition of a community over time. Early colonists often alter the conditions under which later-arriving species grow.

* ■ Primary succession begins at sites that have never been modified by organisms. Review Figure 55.20

► Succession may take place when all or part of the dead body of some organism is decomposed. Review Figure 55.21

Indirect Effects of Interactions among Species

► Indirect effects of species interactions influence many species populations. For example, mice prevent gypsy moth populations from recovering quickly after they defoliate oak trees, thereby allowing the trees to recover. Review Figure 55.22

For Discussion

1. Environmental factors such as temperature, humidity, and salinity, even though they are important in the lives of many organisms, are not considered to be resources. Why not?
2. Kangaroo rats prevent smaller species of rodents from occupying some Sonoran desert habitats. They also reduce populations of seed-harvesting ants, but they do not cause elimination of ants from any habitats. Why can they competitively exclude other rodents, but not ants?
3. On the eastern side of the Sierra Nevada in California, four species of chipmunks occupy adjacent habitats from which they exclude one another by direct aggressive interference. In the San Jacinto Mountains of southern California, three other chipmunk species similarly occupy adjacent habitats, but no interspecific aggression is observed. Each species simply remains in its own habitat. Which of these two assemblages do you think is the older one? Why?
4. What features of predator-prey interactions tend to generate instabilities that lead to fluctuations in the densities of both species? Given that instabilities are expected, what keeps populations of either predator or prey from fluctuating to extinction?
5. Parasites usually have generation times much shorter than those of their hosts. Consequently, they should be able to evolve faster. What prevents them from evolving so fast that they completely overcome the resistance of their hosts and exterminate them?
6. Mimicry of inedible, toxic, or dangerous objects is widespread in nature, but most species are not mimics. Why don't more species evolve to mimic such objects?
7. Wind does not direct pollen toward conspecific stigmas. Given this inefficiency, why are there so many wind-pollinated plants? Similarly, if seeds that land close to the parent plant survive less well than those that are carried farther away, why do so many plants produce seeds lacking dispersal devices?
8. Some direct interactions between two species benefit only one of those species. Give examples of such "one-way" benefits in each of the following cases:
 - a. between two species of plants (give one example of energetic and another example of physical support)
 - b. between a plant and an eater of its leaves
 - c. between a predator and its prey
9. Wood is an abundant food source that has been available for millions of years. Why have so few animals evolved to be able to eat wood?
10. A keystone species exerts a larger influence on the ecological community in which it lives than one would expect given its abundance. What traits are likely to result in a species having such major ecological effects?

56

Ecosystems



IN 1976 THE OUTLET OF SOUTHERN INDIAN Lake in northern Manitoba, Canada, was dammed, raising the lake level 3 meters. Engineers then diverted the Churchill River so that rather than flowing into the lake, it flowed southward across a drainage divide and through a series of hydroelectric generating stations.

Before the dam was built, ecologists studied the lake in detail to assess the likely consequences of raising its level and greatly reducing the flow of river water into it. They predicted that fewer nutrients would enter the lake, but that the reduction would be compensated for by nutrients derived from increased soil erosion along the newly submerged shoreline. Based on their predictions, they believed that the Southern Indian Lake whitefish fishery, the most important commercial fishery in northern Manitoba, would not be harmed.

The ecologists' predictions of the future nutrient status of the lake and amounts of photosynthesis by algae were correct. However, to everyone's surprise, the whitefish fishery was ruined. The greatly increased soil erosion on the new shoreline released large quantities of mercury into the lake. Mercury concentrations in fish in Southern Indian Lake now exceed Canadian safety standards and will probably remain above standard for many years. From 1977 to 1982, Manitoba Hydro, the builder of the dam, subsidized the commercial fishermen, and in 1982, it provided a onetime cash settlement of \$2.5 million Canadian dollars for future losses to the fishermen.

Un expected sur prises commonly follow not only the damming of rivers and lakesTBut mo st attempts to alter ecological systems . Sur-prises arise because the behavior of ecosystems is the result of interactions among many different processes, most of which are only incompletely understood. Ecologists now recognize that mercury pollution often results from the raising of lake levels, but they did not know that in the 1970s. As humans continue to alter Earth's ecological systems, new

surprises confront us each year.

Dams: Some Surprising Aftereffects

Damming rivers and lakes in the expectation of creating benefits for the human population often results in unexpected and unpredictable detrimental effects on the ecosystem.

I



The organisms living in a particular area, such as Southern Indian Lake, together with the physical environment with which they interact constitute an ecosystem. Ecosystems can be recognized and studied at many different spatial scales, ranging from local units—such as a lake—to the entire globe. At the global scale, Earth is a single ecosystem.

The dynamics of ecosystems are the result of the activities of myriad individual organisms, which are influenced by processes in the physical environment. Some of these processes are, in turn, altered by organisms, and some are not. Individuals of the many different species that interact do so by capturing energy and materials, transforming and retaining them, and transferring them to other organisms.

In this chapter we discuss climates on Earth and how they influence ecosystem processes. We then describe patterns of energy flow and the cycles of materials in ecosystems. We will see how knowledge about ecosystems can be used to understand how and why they respond as they do to human-caused disturbances, and how we can learn from our mistakes.

Climates on Earth

The energy of the sun drives the global circulation patterns of air and ocean waters. The warming and cooling of moving masses of air and water explain most of Earth's climatic patterns. Climates, in turn, exert a powerful influence on the distributions, abundances, and evolution of species.



992 CHAPTER FIFTY-SIX

Climates vary greatly from place to place on Earth, primarily because different places receive different amounts of solar energy. The amount of incident solar energy is nearly constant at the equator, but varies dramatically at high latitudes. In this section we will examine how differences in solar energy input determine atmospheric and oceanic circulation.

Solar energy inputs drive global climates

Every place on Earth receives the same total number of hours of sunlight each year—an average of 12 hours per day—but not the same amount of heat. The rate at which heat arrives on Earth per unit of ground area depends primarily on the angle of sunlight. If the sun is low in the sky, a given amount of solar energy is spread over a larger area (and is thus less intense) than if the sun is directly overhead. In addition, when the sun is low in the sky, sunlight must pass through more of Earth's atmosphere, with the result that more of its energy is absorbed and reflected before it reaches the ground. At high latitudes (closer to the poles), there is more variation in both day length and the angle of arriving solar energy over the course of a year than at latitudes closer to the equator. On average, mean annual air temperature decreases about 0.4°C for every degree of latitude (about 110 km) at sea level.

Air temperature also decreases with elevation. The effect of elevation on temperature is due to the properties of gases. As a parcel of air rises, it expands, its pressure drops, and energy is expended in pushing molecules apart. With that loss of energy, the temperature of the air drops. When the parcel of air descends, it is compressed, its pressure rises, the same amount of energy is recovered, and its temperature increases.

When wind patterns bring air into contact with a mountain range, the air rises to pass over the mountains, cooling as it does so. Because cool air cannot hold as much moisture as warm air, clouds frequently form, and moisture is released as rain or snow. On the leeward side of the range, the air, now containing little moisture, descends, warms, and picks up moisture. This pattern often results in a dry area, called a rain shadow, on the leeward side of a mountain range (Figure 56.1).

Global atmospheric circulation influences climates

Earth's climates are strongly influenced by global air circulation patterns. Air rises not only when it crosses mountains, but also when it is heated by the sun. Warm air rises in the tropics, which receive the greatest solar energy input. This air is replaced by air that flows toward the equator from the north and south. That air, in turn, is replaced by air from aloft that descends after having traveled away from the equator at great heights. At roughly 30° north and south latitudes, air that

cooled and lost its moisture when it rose at the equator descends and warms. Many of Earth's deserts, such as the Sahara and the Australian deserts, are located at these latitudes.

At about 60° north and south latitudes, air rises again. Cold, dense air descends at the poles, where there is little

Q Prevailing winds pick up moisture over water bodies.

Q On the windward side of the mountain, air rises and cools, releasing moisture in the form of rain or snow.

On the leeward side of the mountain, air descends, warms, and picks up moisture, which results in little rain.



rp ^ 56.1 A Rain Shadow

Average annual rainfall tends to be lower on the leeward side of a mountain range than on the windward side.

input of solar energy. The black arrows around the edges of Figure 56.2 show these vertical patterns, which are one component of Earth's winds.

The spinning of Earth on its axis influences surface winds because Earth's spinning velocity is rapid at the equator, but relatively slow close to the poles. An air mass at a specific latitude has the same velocity as Earth has at that latitude. As an air mass moves toward the equator, it confronts a faster and faster spin, and its rotational movement is slower than that of Earth beneath it. Similarly, as an air mass moves poleward, it confronts a slower and slower spin, and it speeds up relative to Earth beneath it. Therefore, air masses moving latitudinally are deflected to the right in the Northern Hemisphere and to the left in the Southern Hemisphere. Winds blowing toward the equator from the north and south veer to become the northeast and southeast trade winds, respectively. Winds blowing away from the equator also veer and become the westerlies that prevail at mid-latitudes. The average directions of these surface winds are shown by the blue arrows in Figure 56.2.

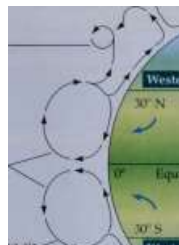
Because Earth's axis is tilted, the amount of solar energy that reaches a given region varies seasonally as Earth orbits the sun. At any given location, the amount of solar energy input is at its maximum at the time of year when the sun is closest to being overhead at noon. The location of greatest solar energy input in the tropics, and the site where the trade winds converge and air rises, is called the intertropical convergence zone (see Figure 56.2). It shifts to the north during the northern summer (southern winter) and to the south during the southern summer (northern winter). Seasonal changes in climate (rainy and dry seasons) in the tropics are associated with the movement of the intertropical convergence zone because whenever an area is within the zone, air rises and heavy rains fall. When the zone is to the north or south of a tropical region, the prevailing winds are trade winds, which seldom yield rain unless forced to rise over mountains.

Jet stream

Westerlies

Rising air

Descending air Jet stream



60° N _ _.

r r r

Forests

30° N Hot deserts

^ ^ ^

Forests

Northeast trades

0° Equator

^ ^ ^N ^

30° S Hot deserts



ECOSYSTEMS 993

56.2 Circulation of Earth's Atmosphere

If we could stand outside Earth and observe its air movements, we would see vertical air movements similar to those indicated by the black arrows and surface winds similar to those shown by the blue arrows. The vertical and horizontal circulation patterns shift to the north during the northern summer and to the south during the northern winter. Thus the intertropical convergence zone is on the equator only twice during each year.

Southeast trades

Global oceanic circulation is driven by winds

The global pattern of wind circulation drives the circulation of ocean water. Ocean water generally moves in the direction of the prevailing winds (Figure 56.3). Winds blowing toward the equator from the northeast and southeast cause water to converge at the equator and move westward until it encounters a continental land mass. At that point the water splits, some of it moving north and some of it moving south along continental shores. The poleward movement of ocean water that has been warmed in the tropics is a major mechanism of heat transfer to high latitudes. As it moves toward the poles, the water veers right in the Northern Hemisphere and left in the Southern Hemisphere. Thus water moves eastward until it encounters another continent and is

deflected laterally along its shores. In both hemispheres, water flows toward the equator along the west sides of continents, continuing to veer right or left until it meets at the equator and flows westward again. The oceans play an important role in world climates, both because their waters move long distances and because water has a high specific heat. The specific heat of a substance is the amount of energy required to raise the temperature of 1 gram of the substance 1°C. For water, this value is 1 cal/g at 15°C. Similarly, 1 gram of water that cools 1°C gives off 1 cal/g. Air and land surfaces have a much lower specific heat. Consequently, in comparison with continents, oceans warm up more slowly in summer because it takes more heat to raise their temperature. Oceans cool off more slowly in winter because more heat must be released to cool them.

56.3 Global Oceanic Circulation

To see that ocean currents are driven primarily by winds, compare the surface currents shown here with the prevailing surface winds shown in Figure 56.2. Deep ocean currents differ strikingly from the surface ones shown here.



West Wind Drift

West Wind Drift

994 CHAPTER FIFTY-SIX

Ecosystem:

Open ocean

Continental shelf

Extreme desert, rock sand Lee

I teseri and semidesert

Tropical rainforesl

Savanna

Culti\ ated land

Boreal Forest (taiga)

Temperate grassland

Woodland and shrubland

Tundra

Tropical seasonal forest

Temperate deciduous forest

Temperate evergreen forest

Swamp and stream

Lake and stream

Estuary

Algal beds and reefs

Upwelling zones

0 10 20 30 40 50 60 (a) Percentage of Earth's surface area

56A Primary Production in Different Ecosystems

The primary production of Earth's ecosystems is measured in several ways, (a) The geographic extent of the different ecosystems. (b) Net annual primary production and (c) the percentage of Earth's total primary production contributed by each ecosystem.

Both wind circulation patterns and the properties of ocean water affect terrestrial climates. At high latitudes, the temperatures of the interiors of large continents fluctuate greatly with the seasons, becoming very cold in winter and ^To TuTsununer, a pattern called a continental climate. The coasts of continents, particularly those on west sides at middle latitudes, where the prevailing winds blow from ocean to land, have maritime climates, with smaller differences between winter and summer temperatures.

The amount and annual pattern of energy input in a region determines the rates at which ecosystem processes operate and the kinds of organisms that live there. Next we will discuss how climates influence the amount of energy that flows through ecosystems.

Energy Flow through Ecosystems

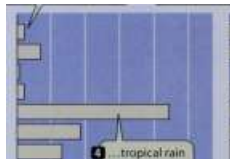
As you learned in Chapter 6, all energy transformations obey the laws of thermodynamics. The first law of thermodynamics states that energy is neither created nor destroyed; that is, the total amount of energy in the universe is constant. The second law of thermodynamics states that when energy is converted from one form to another, some of it becomes unavailable to do work. This law governs patterns of energy flow through ecosystems.

t

Primary production in open ocean is low...

f

...but there is a lot of ocean, so the total % is high.



...tropical rain forest has high production.

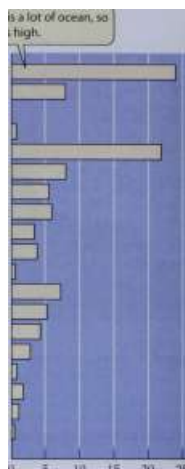
:

i

j

0 500 1000 1500 2000 2500

(b) Average net primary production (grams per m² per year)



(c) Percentage of Earth's net primary production

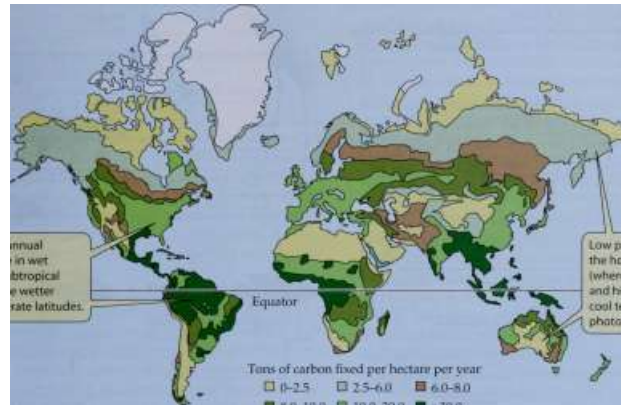
Organisms depend on inputs of energy (in the form of sunlight or high-energy molecules), water, and minerals for metabolism and growth. Except for a few limited ecosystems (some caves, deep-sea hydrothermal systems) in which solar energy is not the main energy source, all energy utilized by organisms comes (or once came) from the sun. Even the fossil fuels—coal, oil, and natural gas—upon which the economy of modern human civilization is based are reserves of captured solar energy locked up in the remains of organisms that lived millions of years ago.

Only about 5 percent of the solar energy that arrives on Earth is captured by photosynthesis. The remaining energy is either radiated back into the atmosphere as heat or consumed by the evaporation of water from plants and other surfaces. The energy that is captured by photosynthesis powers the ecosystem processes. How that energy subsequently passes through a series of organisms is the topic of the next section.

Photosynthesis drives energy flow in ecosystems

Energy flow in most ecosystems originates with photosynthesis. The rate at which plants assimilate energy is called gross primary productivity. Water availability and temperature are major determinants of gross primary productivity. The total amount of energy that plants assimilate by photosynthesis, typically measured over a year, is called gross primary production. The production that remains after subtracting the energy that plants use for their own maintenance (respiration), building tissues, reproduction, and defense is called net primary production (Figure 56.4).

Areas of high annual production are in wet tropical and subtropical regions and the wetter parts of temperate latitudes



Tons of carbon fixed per hectare per year □ 0-2.5 □ 2.5-6.0 □ 6.0-8.0 ■ 8.0-10.0 □ 10.0-30.0 ■ >30.0



Low production characterizes the hot subtropical deserts where moisture is limiting) and high latitudes (where cool temperatures lower photosynthetic rates).

56.5 Net Primary Production of Terrestrial Ecosystems

Variations in temperature and water availability over Earth's land surface affect the annual production of its ecosystems.

The distribution of primary production worldwide reflects the distribution of temperature and moisture on Earth. Close to the equator at sea level, temperatures are high throughout the year and typically the water supply is adequate much of the time. In these climates, highly productive forests thrive. In lower-latitude and mid-latitude deserts, where plant growth is limited by lack of moisture, primary production is low; plants of low stature dominate most landscapes. At higher latitudes, where there is more moisture and trees grow well, primary production is limited by low temperatures during much of the year. Production in aquatic systems is limited by light, which decreases rapidly with depth; by nutrients, which are scarce in open water; and by temperature.

Plants use most of the energy they capture to maintain themselves, to grow, and to reproduce. Some of this energy produces new tissues that can be eaten by herbivores or used by other organisms after the plants die. Because so much of the energy they capture goes to power their own metabolism, however, plants always contain much less energy than the total amount they have assimilated. Only the energy plants do not use to maintain themselves is available to be harvested by animals.

"The global distribution of net primary production is shown in Figure 56.5.

Energy flow through a series of organisms

Because energy flows through ecosystems when organisms eat one another, it is useful to group organisms according to their source of energy. The organisms in an ecosystem that obtain their energy from a common source constitute a trophic level (Table 56.1). Organisms at a particular trophic level occupy a position in an ecosystem that is determined

JO, 1 The Major Trophic Levels

TROPHIC LEVEL

SOURCE OF ENERGY

EXAMPLES

Photosynthesizers

(primary producers) Herbivores

Primary carnivores Secondary carnivores Omnivores Detritivores (decomposers)

Solar energy

Tissues of primary producers

Herbivores Primary carnivores Several trophic levels Dead bodies and waste products of other organisms

Green plants, photosynthetic bacteria and protists

Termites, grasshoppers, water fleas, anchovies,

deer, geese Spiders, warblers, wolves, copepods Tuna, falcons, killer whales Humans, opossums, crabs, robins Fungi, many bacteria, vultures, earthworms

996 CHAPTER FIFTY-SIX

56.6 A Food Web Diagram for a Lake

This food web diagram summarizes the major predator-prey interactions within Gatun Lake, Panama. The arrows show who eats whom.

b\ the number of steps through which energy passes to reach them. Photosynthetic plants get their energy directly from sunlight. Collectively they constitute the trophic level called photosynthesizers or primary producers. They produce the energy-rich organic molecules upon which nearly all other organisms feed.

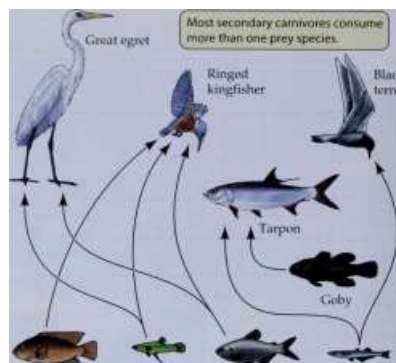
All other organisms consume, either directly or indirectly, the energy-rich organic molecules produced by photosynthetic organisms. Organisms that eat plants constitute the trophic level called herbivores. Organisms that eat herbivores are called primary carnivores. Those that eat primary carnivores are called secondary carnivores, and so on. Organisms that eat the dead bodies of organisms or their waste products are called detritivores or decomposers. The many organisms that obtain their food from more than one trophic level are called omnivores."

A sequence of linkages in which a plant is eaten by an herbivore, which is in turn eaten by a primary carnivore, and so on, is called a food chain. Food chains are usually interconnected in a food web, because most species in a community eat and are eaten by more than one other species."

A food web diagram is a useful summary of predator-prey interactions within a community. A simplified food web diagram (not including detritivores) for Gatun Lake, Panama, is shown in Figure 56.6. A complete food web diagram, showing the position of every species in a community, would be confusingly complex because most biological communities contain so many species. Therefore, similar species, especially those at lower trophic levels, are usually lumped together, as they are in the diagram of the Gatun Lake food web.

Sun

Most secondary carnivores consume more than one prey species.



Cichlosoma bartoni

Poeciliidae (Gambusia)

Characinidae (Terra)

Melaniris chagresi



C. bartoni feeds on algae.

Three groups of fish eat insects.



Filamentous green algae



Mosquito larvae

Zooplankton

Small phytoplankton

The lowest trophic levels contain many species of phytoplankton and zooplankton.

Process:

J Photosynthesis

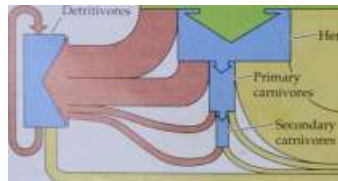
I—I Digestion, assimilation, '—' and growth

j Excretion and death [] Respiration

I

The greatest amount of energy is lost to respiration and is unavailable to organisms.

Photosynthetic organisms



Herbivores

Respiration

Much energy is lost between trophic levels

The energy that organisms use to maintain themselves is dissipated as heat, a form of energy that cannot be used by other organisms. For this reason, only a small portion of the energy captured at one trophic level is available to organisms at the next higher level. The energy content of an organism's net production—its growth plus reproduction—is available to organisms at the next trophic level (Figure 56.7).



56.7 Energy Flow through an Ecosystem

In this diagram, the width of each channel is roughly proportional to the amount of energy flowing through it. The arrows indicate directions of energy flow.

The efficiency of energy transfer through food webs depends on:

- The fraction of net production at one trophic level that is consumed by organisms at the next level
- How those organisms divide the ingested energy between production and maintenance ~"

Birds and mammals have very low production efficiencies because they expend so much energy maintaining constant high body temperatures. Herbivores are less efficient than carnivores because plant tissues generally take more energy to digest than animal tissues do, but because of the low efficiency of energy transfer between trophic levels, a given amount of primary production can support many more herbivores than carnivores.

The amount of energy reaching a trophic level is determined by net primary production in the ecosystem and by the efficiencies with which food energy is converted to biomass (the total weight of organisms) at the trophic levels below it. To show how energy decreases in moving from lower to higher trophic levels, ecologists construct diagrams called pyramids of energy. A pyramid of biomass, which shows the mass of organisms existing at different trophic levels, illustrates the amount of biomass that is available at a given moment in time for organisms at the next trophic level (Figure 56.8).

Pyramids of energy and biomass for the same ecosystem usually have similar shapes, but sometimes they do not. The shapes depend on the dominant organisms and how they allocate their energies. Terrestrial ecosystems may differ strikingly in patterns of energy flow depending on the life forms of the dominant plants.

In grassland ecosystems, for example, where plants produce few hard-to-digest woody tissues, animals are able to consume most of the annual production of plant tissues each year. In grasslands, mammals—wild or domestic—may consume 30-40 percent of the annual aboveground net primary production. Insects may consume an additional 5-15 percent. Soil organisms, primarily nematodes, may consume 6-40 percent of the belowground biomass (Figure 56.8a).

In forest ecosystems, the dominant plants allocate a great deal of their energy to forming wood, which accumulates at high rates in growing forests. Wood, which is constructed of difficult-to-digest material (such as cellulose and lignin), is rarely eaten unless a plant is diseased or otherwise weakened. In most forests, leaves fall to the ground relatively undamaged at the end of the growing season. Although there are outbreaks of defoliating insects in forests, browsing rates are generally so low that ecologists often ignore losses to herbivores when calculating forest production (Figure 56.8b).

In most aquatic communities, on the other hand, the dominant photosynthesizers are bacteria and protists.

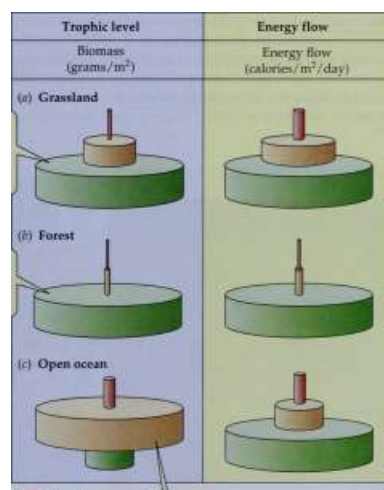
These organisms have such high rates of cell division that a small biomass of photosynthesizers can feed a much larger biomass of herbivores, which grow and reproduce much more slowly. This pattern can produce an inverted pyramid of biomass, even though the pyramid of energy for the same ecosystem has the typical shape (Figure 56.8c).

Much of the energy ingested by organisms is converted to biomass that is eventually consumed by detritivores (see Figure 56.7). Detritivores transform the remains and waste products of organisms (detritus) into carbon dioxide, water, and free mineral nutrients that can be taken up by plants again. If there were no detritivores, most nutrients would eventually be tied up in dead bodies, where they would be unavailable to plants. Therefore, continued ecosystem productivity depends on rapid decomposition of detritus.

Under the warm, wet conditions found in tropical forests, detritus is decomposed within a few weeks or months, and no litter accumulates on the soil surface. Rates of decomposition are slower under colder, drier, or highly acidic conditions, such as those in many coniferous forests. At high altitudes and latitudes, decomposition of leaf litter

Most of the biomass in a grassland is found in the green plants, and most of the energy flows through them.

In forests, the majority of biomass is tied up in wood and is mostly unavailable to herbivores.



| Carnivores | Herbivores | Producers

A marine community produces an inverted pyramid of biomass. The producers are unicellular algae, which divide so rapidly that a small biomass can support a much larger biomass of herbivores.

v.

56.8 Pyramids of Biomass and Energy

Ecosystems can be compared in terms of the amount of material present in organisms at different trophic levels (left) and in terms of energy flow (right).

998 CHAPTER FIFTY-SIX

It can take decades, and decomposition of tree trunks may take more than a century.

Some ecosystems are not powered by direct sunlight

Most ecosystems are powered by sunlight falling directly on them, but some depend upon sunlight that falls elsewhere. For example, marine ecosystems deeper than the level at which enough light penetrates for photosynthesis depend on biomass produced in the well-lit zone above them. The productivity of most deep-sea ecosystems is low because only small amounts of detritus descend through the water column to reach them.

Some deep-sea ecosystems are totally independent of sunlight. The most striking are those around hydrothermal vents associated with seafloor spreading zones. The energy base of these ecosystems is chemosynthesis by sulfur-oxidizing

bacteria. These bacteria obtain energy by oxidizing hydrogen sulfide in the hot water emitted from the vents. Most other organisms in these ecosystems, such as vestimentiferans, live directly or indirectly on these sulfur-oxidizing bacteria.

Ecologists recently discovered a cave ecosystem in southern Romania that is now fed by bacteria that fix inorganic carbon by using hydrogen sulfide as an energy source. Chemoautotrophic production by these bacteria is the food base for 48 species of cave-adapted terrestrial and aquatic invertebrates. However, most cave ecosystems depend on imported photosynthetically produced organic matter.

(«)

Humans manipulate ecosystem productivity

Through agriculture, humans exploit ecosystems by replacing species of low economic value with species of high value. We do this by manipulating ecosystems so as to increase the yield of products useful to us. Agriculture has several intricately intertwined components:

- ▶ We eliminate competition between crops and unwanted plants by cultivating and by applying herbicides.
- ▶ We reduce competing herbivores and disease-causing organisms, usually by applying toxic chemicals.
- ▶ We augment photosynthesis by adding nutrients (fertilizing) and water (irrigating).
- ▶ We develop special high-yielding strains of plants that respond to additional fertilizer by increasing their growth or reproductive rates.

* —

All these manipulations must work together, because "miracle" strains of crops typically do not yield more than other strains unless they are provided with fertilizers and protected from competitors, herbivores, and pathogens. Agriculture also depends on energy from outside the system for cultivation and harvesting. In modern agriculture, this energy comes from fossil fuels (Figure 56.9).

Modern agricultural ecosystems have spectacularly increased food production per hectare, but they have also created problems. Herbicides and insecticides have polluted lakes, rivers, and groundwater in most industrialized countries. Many agricultural pests have evolved resistances to pesticides. New methods of pest control, collectively known as integrated pest management (IPM), are becoming more common. IPM combines chemical applications with cultural practices—crop rotation, mixed plantings of crop plants, and mechanical tillage of the soil—and biological methods—development of pest-resistant strains of



56.9 Agriculture Requires Energy Input

(a) In traditional agriculture, people and domesticated animals supply most of the energy, as in this Indian rice paddy, (b) Modern agriculture is based on the use of toxic chemicals and high rates of consumption of fossil fuels during site preparation, growth of the crop, and at harvest time. The large machines harvesting this wheat field are typical of much modern agriculture.

00

2f*



xT~



crops, use of natural predators and parasites, and use of chemical attractants, such as pheromones—to control insect herbivores. The reduced use of toxic chemicals avoids most pollution problems and reduces the chance that pests will evolve resistance to pesticides.

Cycles of Materials through Ecosystem Compartments

As we have just seen, energy flows through ecosystems according to the second law of thermodynamics. At each transformation, much of it is dissipated as heat, a form that cannot be used by organisms to power their metabolism. Chemical elements, on the other hand, are not lost when they are transferred among organisms; they cycle repeatedly through organisms and the physical environment. The carbon and nitrogen atoms of which life is composed today are the same atoms that made up dinosaurs, insects, and trees in the Mesozoic era. The amounts of carbon, nitrogen, phosphorus, calcium, sodium, sulfur, hydrogen, oxygen, and other chemical elements on Earth do not change, but the quantities that are available to organisms are strongly influenced by how organisms get them, how long they hold onto them, and what they do with them while they have them.

To understand the cycling of elements, it is convenient to divide the global ecosystem into four compartments: (1) oceans, (2) fresh water, (3) land, and (4) atmosphere. The physical environments in each compartment and the types of organisms living there are different. Therefore the amounts of elements found in the different compartments, what happens to those elements, and the rates at which they enter and leave the compartments differ strikingly. After we have described these compartments, we will consider them together to illustrate how elements cycle through the global ecosystem.

Oceans receive materials from the land and atmosphere

Oceans receive chemical elements from land as runoff from rivers. The immediate receivers of materials from land are often fresh waters and the atmosphere, but, on time scales of hundreds to thousands of years, oceans receive most of those materials, including those produced by human activity. Because of their huge size, and because oceans exchange materials with the atmosphere only at their surface, oceans respond very slowly to outside inputs.

Elements that enter the oceans from other compartments gradually sink to the seafloor and remain there, unless

something happens to bring them back to the surface. Most elements remain in the bottom sediments until they are elevated above sea level by movements of Earth's crust. This process may take many millions of years. For this reason, concentrations of mineral nutrients are very low throughout the ocean. Oxygen, however, is usually present at all depths, because even slow mixing suffices to replenish the oxygen consumed by the respiration and decomposition of the few organisms that live in the nutrient-poor ocean waters.

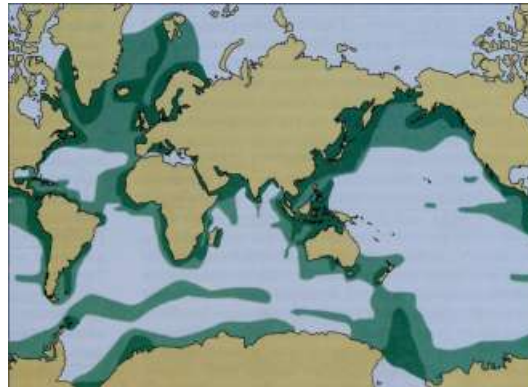
Except on the relatively shallow continental shelves, ocean waters mix very slowly and are strongly stratified by depth. Typically there is a well-defined depth zone—a thermocline—at which temperatures drop abruptly. Some elements are stirred to the surface by cool bottom water that rises up well. Much upwelling occurs near the coasts of continents where offshore winds push surface waters away from shore (Figure 56.10). Waters in these zones of upwelling are rich in nutrients, and most of the world's great fisheries

" $r' - 7^{\circ}$ ■ -.

are concentrated there.

Lakes and rivers contain only a small fraction of Earth's water

Lakes and rivers contain much less water than oceans do, and because these bodies of water are relatively small, most mineral nutrients entering them are not buried in bottom sediments for long periods of time. Some mineral nutrients enter fresh waters in rainfall, but most are released by the weathering of rocks and are carried to lakes and rivers via



Primary production (mg of carbon per m² per day)

□ <150 | 1150-250 H >25 °

56.10 Primary Production is High in Zones of Upwelling

Primary production in the oceans is highest near continents where surface waters, driven by prevailing winds, move offshore and are replaced by cool, nutrient-rich water upwelling from below.

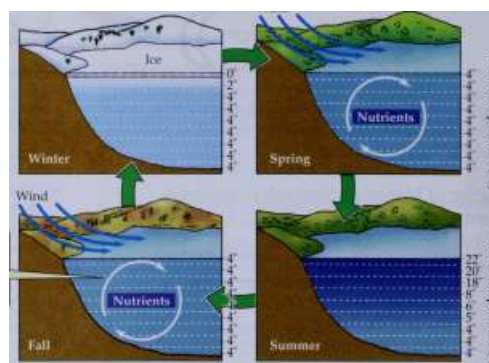
1000 CHAPTER FIFTY-SIX

56.11 Annual Temperature and Oxygen Cycles in a Temperate Lake

These vertical temperature and oxygen profiles are typical of temperate zone lakes that freeze in winter.

Wind

Turnovers occur in the spring and fall and allow nutrients and oxygen to become evenly distributed in the water column.



g groundwater (the water that resides in soil and rocks) or by surface flow.

After entering rivers, mineral nutrients are usually carried rapidly to lakes or to the oceans. In lakes they are taken up by organisms and incorporated into their cells. These organisms eventually die and sink to the bottom, where decomposition of their tissues consumes the oxygen. Surface waters of lakes thus quickly become depleted of nutrients, while deeper waters become depleted of oxygen. However, this stratification process is countered by vertical movements of water—turnover—that bring nutrients and dissolved CO₂ to the surface and oxygen to deeper water. Wind is an important agent of turnover in shallow lakes, but in deeper lakes it usually mixes only surface waters.

Lakes in temperate regions turn over because water is most dense at 4°C; above and below that temperature it expands (Figure 56.11). In spring, the sun warms the surface layer of a lake. The depth of the warm layer gradually increases as spring and summer progress. However, there is still a well-defined thermocline where the temperature drops abruptly to about 4°C. Only if the lake is shallow enough to warm to the bottom does the temperature of the deepest water rise above 4°C.

In autumn, as the surface of the lake cools, the cooler surface water, which is denser than the warmer water below it, sinks, and is replaced by warmer water from below. This process continues until the entire lake has reached 4°C. At this point, the

density of the water is uniform throughout the lake, and even modest winds readily mix the entire water column. As colder weather then cools the surface water below 4°C, that water becomes less dense than the 4°C water below it. Therefore, it floats at the top. Another turnover occurs in spring, when the surface layers above the thermocline warm to 4°C and the water column, again being of uniform density throughout, is easily mixed by wind.

Deep tropical and subtropical lakes may be permanently stratified because they never become cool enough to have uniformly dense water. Their bottom waters lack oxygen because decomposition quickly depletes any oxygen that

reaches them. However, many tropical lakes are overturned at least periodically by strong winds so that their deeper waters are occasionally oxygenated. Arctic lakes turn over only once each year.

The atmosphere regulates temperatures close to Earth's surface

The atmosphere is a thin layer of gases surrounding Earth. About 80 percent of the mass of the atmosphere lies in its lowest layer, the troposphere, which extends upward from Earth's surface about 17 km in the tropics and subtropics, but only about 10 km at higher latitudes. Most global air circulation takes place within the troposphere, and virtually all atmospheric water vapor is located there.

The stratosphere, which extends upward from the top of the troposphere to about 50 km above Earth's surface, con-

tains about 99 percent of the remaining atmospheric mass, but it is extremely dry. Materials enter the stratosphere from the troposphere near the equator, where air rises to high altitudes. These materials tend to remain in the stratosphere for a relatively long time because stratospheric air circulation is horizontal. Ozone (O_3) in the stratosphere absorbs most incoming short-wavelength ultraviolet radiation, shielding organisms from its damaging effects.

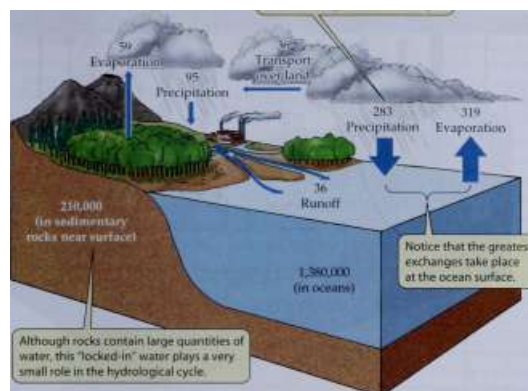
The atmosphere is composed of 78.08 percent nitrogen as N_2 , 20.95 percent oxygen, 0.93 percent argon, and 0.03 percent carbon dioxide. It also contains traces of hydrogen gas, neon, helium, krypton, xenon, ozone, and methane. The atmosphere contains Earth's biggest pool of nitrogen and large supplies of oxygen. Although carbon dioxide constitutes a very small fraction of the atmosphere, it is the source of the carbon used by terrestrial photosynthetic organisms.

The atmosphere plays a decisive role in regulating temperatures at and close to Earth's surface. Without an atmosphere, the average surface temperature of Earth would be about -18°C, rather than the actual +17°C. The atmosphere is relatively transparent to visible light, but it traps a large part of the outgoing infrared radiation (heat) that is emitted by Earth. It traps heat at Earth's surface in a way analogous

The numbers give the relative amounts of water (expressed as units of 10^{18} g) held or exchanged.

ECOSYSTEMS

1001



Although rocks contain large quantities of water, this "locked-in" water plays a very small role in the hydrological cycle.

to the glass in a greenhouse, which is why this phenomenon is called the greenhouse effect. Water vapor, carbon dioxide, and ozone are especially important transmitters of infrared radiation. That is why, as we will see below, elevated concentrations of atmospheric carbon dioxide caused by human activities may lead to major climatic changes.

Land covers about one-fourth of Earth's surface

About one-fourth of Earth's surface, most of it in the Northern Hemisphere, is currently above sea level. Most of this land is covered by a layer of soil. Even though the global supply of nutrients is constant, regional and local deficiencies strongly affect ecosystem processes on land. Such deficiencies occur because, unlike their behavior in air and water, elements on land move slowly, and they usually move only short distances.

The terrestrial compartment is connected to the atmospheric compartment by terrestrial organisms that take chemical elements from and release them to the air. Chemical elements in soils are carried in solution into the ground-water and eventually into rivers and oceans where they are lost to organisms until geological processes raise marine sediments above sea level, and a new cycle of erosion and weathering begins. The type of soil that forms in an area and the elements it contains depend on the underlying rock, as well as on climate, topography, the organisms living there, and the length of time that soil-forming processes have been acting. Very old soils contain fewer nutrients than most young soils.



Biogeochemical Cycles

The chemical elements organisms need in large quantities—carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur—cycle through organisms to the physical environment and back again.

56.12 The Global Hydrological Cycle

The numbers show the relative amounts of water (expressed as units of 10^{18} g) held in or exchanged annually by ecosystem compartments. The widths of the arrows are proportional to the sizes of the fluxes.

environment and back again. The pattern of movement of a chemical element through organisms and compartments of the physical environment is called its biogeochemical cycle. Some chemical elements circulate continually, but large quantities of other elements are temporarily lost from circulation through deposition in deep-sea sediments.

Each chemical element used by organisms has a distinctive biogeochemical cycle whose properties depend on the physical and chemical nature of the element and how organisms use it. All chemical elements cycle quickly through organisms because no individual, even of the longest-lived species, lives very long in geological terms. Chemical elements, such as carbon and nitrogen, that exist in the atmosphere as a gas cycle faster than nongaseous elements. After discussing the movements of water, we discuss the cycling of the most abundant chemical elements in organisms.

Water cycles through the oceans, fresh waters, atmosphere, and land.

The cycling of water through the four ecosystem compartments is known as the hydrological cycle (Figure 56.12). Although water is a compound, not an element, we describe its cycle here together with those of individual elements because of its importance to life.

The hydrological cycle is driven by the evaporation of water, most of it from ocean surfaces. Some water returns to the oceans as precipitation, but much less falls back on the oceans than is evaporated from them. The remaining evaporated water is carried by winds over the land, where it falls as rain or snow.

Water also evaporates from soils, from freshwater lakes and rivers, and from the leaves of plants (transpiration), but the total amount evaporated is less than the amount that falls on land as precipitation. The excess water eventually returns to the oceans via rivers, coastal runoff, and subterranean flows.

Organisms profoundly influence the carbon cycle.

Organisms are triumphs of carbon chemistry. To survive, they must have access to carbon atoms. Nearly all the carbon in organisms comes from carbon dioxide (CO_2) in the atmosphere or dissolved carbonate ions (HCO_3^-) in water. In the cells of some bacteria, photosynthetic protists, and the leaves of plants, carbon is incorporated into organic molecules.

t

Q The two large reservoirs of carbon are carbon-containing minerals in rocks (including fossil fuels)...



1002 CHAPTER FIFTY-SIX

56.13 The Global Carbon Cycle

The numbers show the quantities of carbon (expressed as units of 10^{15} g) in organisms and in ecosystem compartments, and the amounts that move annually between them.

Deforestation:

(1-2)⁴

(yul es by pho tos ynthe sis. All organisms in Other groups get their carbon by consuming other organisms, their remains, or their waste products.

Although marine organisms contain a very little of Earth's total carbon, they have a profound influence on the distribution of carbon in the oceans. They convert soluble carbonate ions in seawater into insoluble ocean sediments by depositing carbon in their shells and skeletons, which eventually sink to the bottom.

Biological processes on land move carbon between the atmospheric and terrestrial compartments, removing it from the atmosphere during photosynthesis and returning it to the atmosphere during respiration (Figure 56.13). Growing plants at mid- and high latitudes in the Northern Hemisphere incorporate so much carbon into their bodies that they reduce the concentration of atmospheric CO_2 , from about 350 parts per million in winter to 335 parts per million in midsummer. This carbon is released back into the atmosphere by decomposition in autumn.

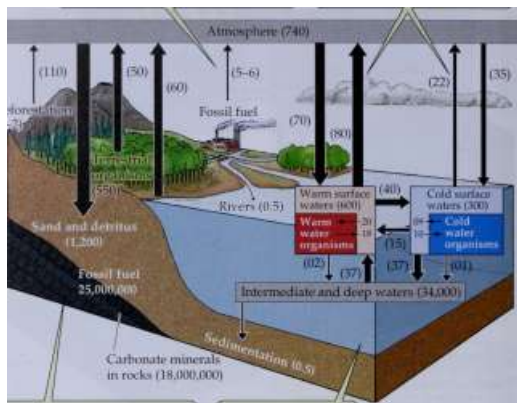
At times in the remote past, large quantities of carbon were removed from the cycling when organisms died in large numbers and were buried in sediments lacking oxygen. In such environments, detritivores do not reduce organic carbon to carbon

dioxide. Instead, organic molecules accumulate and eventually are transformed into oil, natural gas, coal, or peat (decomposed remains of plants in high-latitude bogs). Humans have discovered and used these deposits, known as fossil fuels, at ever increasing rates during the past 150 years. As a result, carbon dioxide, the final product of burning these fuels, is being released into the atmosphere faster than it is being transferred to the oceans and incorporated into terrestrial biomass (Figure 56.14).

Based on a variety of calculations, atmospheric scientists believe that 150 years ago, before the Industrial Revolution, the concentration of atmospheric CO₂ was probably about 265 parts per million. Today it is 350 parts per million. This increase has been caused primarily by combustion of fossil fuels and secondarily by the burning of forests. If current trends in both these activities continue, atmospheric CO₂ is expected to reach 580 parts per million by the middle of the twenty-first century. This CO₂ will eventually be transferred to the oceans and deposited in sediments as calcium carbonate (CaCO₃), but the rate of transfer is much slower than the rate at which humans are introducing CO₂ into the atmosphere.

Atmospheric CO₂ is the immediate source of carbon for terrestrial organisms.

The widths of the arrows are proportional to the size of the flux.



o...,in Tintin

d dissolved carbon the oceans.

Enough carbon is being released by the burning of fossil fuels to alter the heat balance of Earth. As we saw above, carbon dioxide is one of the components of the atmosphere that traps infrared radiation at Earth's surface. Scientists have measured the concentration of CO₂ in air trapped in the Antarctic and Greenland ice caps during the last Ice Age—between 15,000 and 30,000 years ago—when the climate was much colder. The CO₂ concentration then was as low as 200 parts per million. During a warm interval that occurred some 5,000 years ago, it may have been slightly higher than it is today. This long-term record, which

•a "x o

•3

c

o

X>

1-4

U

Oh

360

QEach yearCO₂ concentrations rise during the winter, when respiration exceeds photosynthesis.

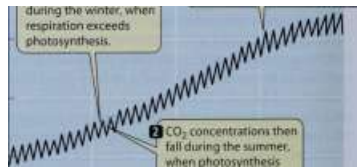
.2 340

320

300

I

The steady rise in overall concentrations is very apparent.



CO₂ concentrations then fall during the summer, when photosynthesis exceeds respiration.

1960

1970

1980 Year

1990

2000

H



56.14 Atmospheric Carbon Dioxide Concentrations Are Increasing

These carbon dioxide concentrations, expressed as parts per million by volume of dry air, were recorded on top of Mauna Loa, Hawaii.

EXPERIMENT

Question: What effect do CO₂ levels have on a community of soil organisms?

ECOSYSTEMS

1003

METHOD

1. Establish multiple plots containing the same communities of organisms. Maintain half of the units at experimentally high levels of atmospheric CO₂, the others at normal atmospheric levels.
2. Maintain communities for three generations (9 months).
3. Measure abundances of three species of collembolans (springtails), prominent members of the community.

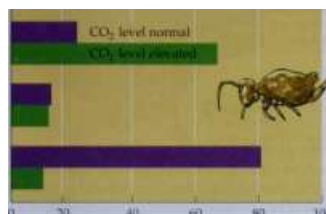
RESULTS With elevated CO₂, species 1 became more prevalent, while species 2 remained the same and species 3 dramatically decreased.

Species 1

Species 2

Species 3

CO₂ level normal



20 40 60

Proportion in soil community

Conclusion: High levels of CO₂ dramatically changed the species composition of the experimental communities.

56.15 Increased Atmospheric CO₂ Concentrations Alter Soil Communities

After 9 months of exposure to elevated CO₂ concentrations, the relative abundances of three collembolan insect species (spring-tails) differed dramatically from those in communities exposed to normal atmospheric CO₂ concentrations.

It demonstrates a relationship between climate and atmospheric CO₂ levels, is a major reason why scientists expect global

warming as atmospheric CO₂ levels continue to increase.

A doubling of atmospheric CO₂ would shift climate zones toward the poles. Complex computer models predict there would probably be droughts in the central regions of continents while precipitation would increase in coastal areas.

Current evidence indicates such warming is already underway. The average global temperature has risen steadily over the past 20 years, and the sheets of sea ice surrounding the polar land masses have become smaller and thinner. The glaciers have risen by an average of a few centimeters worldwide, and some islands, such as Bermuda, have seen even greater effects. If global warming were to result in the melting of the Greenland and Antarctic ice caps, rising sea levels could flood coastal cities and agricultural lands.

In addition to studying the potential effects of global warming on ecosystem processes, ecologists are investigating

the direct effects of elevated CO₂ concentrations on ecosystems. Increases in CO₂ affect plants directly because CO₂ is the source of carbon for photosynthesis. Their effects on other organisms are indirect.

To determine how elevated concentrations of atmospheric CO₂ might influence soil biota, ecologists established and maintained sixteen 1-m² experimental plots, into which they introduced the same community of plants, herbivores, carnivores, and soil microorganisms. All sixteen plots were maintained under the same conditions, except that CO₂ levels were allowed to vary naturally in half of them but were kept high in the other half. After three plant generations, the abundance and species composition of soil microorganisms were about the same under the two conditions. However, populations of cellulose-decomposing fungi increased dramatically under the elevated CO₂ condition, and the species composition also changed. Populations of springtails—wingless insects that eat fungi—also increased, and different species dominated the biota (Figure 56.15). The sequence of these changes appeared to be:

- ▶ increased plant photosynthesis →
- ▶ increased transport of carbon belowground →
- ▶ increased dissolved organic carbon →
- ▶ changes in soil fungal assemblages →
- ▶ changes in abundances and species composition of springtails.

To ecologists, these changes are striking. However, these experiments inform us only about relatively short-term changes. Other experiments are being run to assess long-term changes in the functioning of ecological systems in response to elevated atmospheric concentrations of CO₂.

Few organisms can use elemental nitrogen

Although nitrogen gas (N₂) makes up about 78 percent of the atmosphere, it cannot be used by most organisms in this form. It can be converted into biologically useful forms only by a few species of bacteria and cyanobacteria, which convert N₂ into ammonia by a process called nitrogen fixation (see Chapter 36). So, despite the abundance of N₂, usable nitrogen is often in short supply in ecosystems.

Why don't nitrogen-fixing organisms increase in numbers so that nitrogen is no longer a limiting resource? Fixing nitrogen is energetically expensive; as a result, nitrogen-fixing organisms often lose out in competition with non-fixers when nitrogen becomes more readily available. Nitrogen tends to be lost rapidly from ecosystems by leaching, volatilization of ammonia, and denitrification (the return of nitrogen to the atmosphere as nitrogen gas, N₂ by some organisms), but is released only slowly by decomposition of detritus.

Organisms called nitrifiers neither take up nitrogen gas directly from the atmosphere nor respire nitrogen back to the atmosphere. Instead, they convert organic molecules containing nitrogen to inorganic molecules. This process is carried out in several stages by different organisms. Most of the resulting "nitrogen-containing compounds, such as nitro-

o-

"D

1004 CHAPTER FIFTY-SIX

56.16 The Global Nitrogen Cycle

The numbers show the quantities of nitrogen (expressed as units of 10⁹ kg) in organisms and in ecosystem compartments, and the amounts that move annually between compartments. The widths of the arrows are proportional to the sizes of the fluxes.

The several stages of inorganic nitrogen are nitrate (NO₃⁻), nitrite (NO₂⁻), and ammonium (NH₄⁺).

Nitrate or ammonia, are taken up by plants. This movement of nitrogen among organisms accounts for about 95 percent of all nitrogen flux on Earth (Figure 56.16).

Organisms drive the sulfur cycle

Emissions of volatile sulfur dioxide (SO_2) and hydrogen sulfide (H_2S) from volcanoes and fumaroles (vents for hot gases) are the primary significant natural nonbiological fluxes

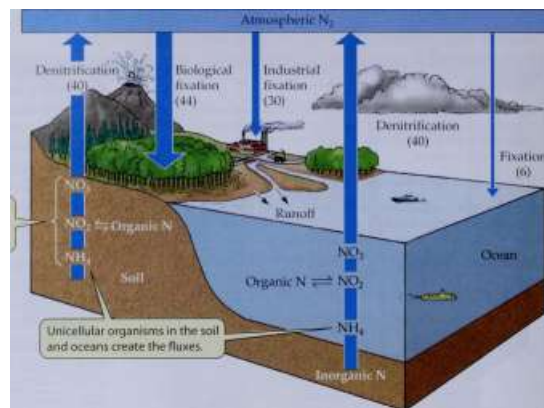
of sulfur. These emissions release, on average, between 10 and 20 percent of the total natural flux of sulfur to the atmosphere, but they vary greatly in time and space. Large volcanic eruptions spread great quantities of sulfur over broad areas, but they are rare events.

Volatile sulfur compounds are also emitted by both terrestrial and marine organisms. Certain marine algae produce large amounts of dimethyl sulfide (CH_3SCH_3), which accounts for about half of the biotic component of the sulfur cycle. The other half is produced by terrestrial organisms.

Sulfur is apparently always abundant enough to meet the needs of living organisms. It also plays an important role in global climate patterns. Even if air is moist, clouds do not form readily unless there are small particles around which water can condense. Dimethyl sulfide is the major source of such particles. Therefore, increases or decreases in sulfur emissions can change cloud cover and hence climate.

Humans have altered the nitrogen and sulfur cycles, largely through the burning of fossil fuels. An important regional effect of this alteration is acid precipitation—rain or snow whose pH is lowered by the presence of sulfuric acid (H_2SO_4) and nitric acid (HNO_3). These acids enter the atmosphere, primarily from industrial smokestacks and automobile emissions, and may travel hundreds of kilometers before they settle to Earth in precipitation or as dry particles.

Acid precipitation now characterizes all major industrialized countries and is particularly widespread in eastern North America and Europe. The normal pH of precipitation in New England is about 5.6, but precipitation there now averages about pH 4.1, and there are occasional storms with a precipitation pH as low as 3.0. Precipitation with a pH of about 3.5 or lower causes direct damage to the leaves of plants and reduces photosynthetic rates. Fortunately, as a result of the establishment of a flexible regulatory system



under the 1990 Clean Air Act Amendments, precipitation in much of the eastern United States is less acid today than it was 15 years ago, primarily because of reductions in emissions of sulfur (Figure 56.17).

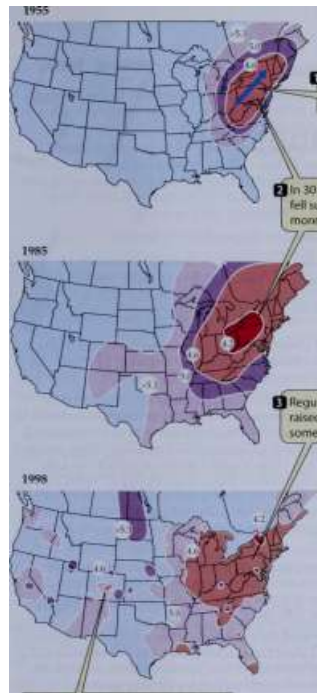
Ecologists in Canada studied the effects of acid precipitation by adding enough sulfuric acid to two small lakes to reduce their pH from about 6.6 to 5.2. In both lakes, nitrifying bacteria

ceased to function. As a result, the nitrogen cycle was blocked and ammonium accumulated in the water. When the ecologists stopped adding acid to one of the lakes, its pH increased to 5.4, and nitrification resumed. After about 1-year, the pH of the lake returned to its original value. These experiments show that lakes are very sensitive to acidification, but can recover quickly when pH returns to normal values.

The phosphorus cycle has no gaseous phase

The phosphorus cycle differs from the other biogeochemical cycles discussed in this section in that it lacks a gaseous phase. Some phosphorus is transported on dust particles, but in general the atmosphere plays a very minor role in the phosphorus cycle. Phosphorus exists mostly as phosphate (PO_4^{3-}) or similar compounds. Most phosphate deposits are of marine origin. On land, phosphorus becomes available through the slow weathering and dissolution of rocks and minerals.

Organisms need phosphorus as a component of the energy-rich molecules involved in cellular metabolism. Phosphorus is often a limiting nutrient in soils and lakes. That is why phosphate is a component of most fertilizers, and why adding phosphate to lakes causes marked increases in their biological productivity. The extra phosphorus from fertilizers and detergents that enters lakes through runoff allows algae and bacteria to multiply, forming blooms that turn water green. The decomposition of the extra biomass produced by this increased biological activity consumes all the oxygen in the lake, and anaerobic organisms come to dominate the sediments. These anaerobic organisms do not



Because of prevailing winds, acid rains affect areas far from the pollution sources.

ECOSYSTEMS 1005

56.17 Acid Precipitation is Decreasing in the Eastern United States

Thanks to emission controls, precipitation in many parts of the eastern United States is less acid than it was two decades ago. The figure shows the regional average pH of precipitation.

In 30 years, pH of precipitation fell substantially (became more acidic).

fj Regulation of emission sources has raised the pH of precipitation in some areas of the Northeast...

f

... but in many areas of the West, precipitation is becoming more acidic.

break down organic compounds all the way to carbon dioxide. Many of the end products of their activities have unpleasant odors.

Lake Erie is an example of such a ^utrophic (enriched) lake. Two hundred years ago it had only moderate levels of photosynthesis and clear, oxygenated water. Today more than 15 million people live in the Lake Erie basin. Nearby cities pour more than 250 billion liters of domestic and industrial wastes into the lake annually. The entire basin is intensely farmed and heavily fertilized.

In the early part of the twentieth century, nutrients in the lake increased greatly, and algae proliferated. At the water filtration plant in Cleveland, algae increased from 81 per milliliter in 1929 to 2,423 per milliliter in 1962. Algal blooms and populations of bacteria also increased. Numbers of the human fecal bacterium *Escherichia coli* increased enough to cause the closing of many of the lake's beaches as health

As a result, the amount of phosphate added to Lake Erie has decreased more than 80 percent from the maximum level, and phosphorus concentrations in the lake have declined substantially. ^Tre~deeper waters of Lake Erie still become poor in oxygen during the summer months, but the rate of oxygen depletion is declining. Algal blooms have decreased, as have populations of small fishes that feed on algae.

The rate at which a eutrophic lake recovers depends on the replacement rate of its waters. Because water flows slowly through Lake Erie, it will take many years for the lake to recover from the heavy pollutant loads it has received. By contrast, the water in Lake Washington, a smaller lake adjacent to the city of Seattle, is replaced every 3 years. When sewage was diverted away from Lake Washington, the lake returned to its former condition within a decade.

Humans influence other biogeochemical cycles

In addition to modifying the great natural biogeochemical cycles we have just described, human activities are increasing the cycling of elements such as lead and creating cycles of synthetic chemicals, such as DDT. These changes are large enough to cause serious environmental problems. However, the results of experiments show [■]tha ecosystems have the capacity to recover from many disturbances*! the alterations have not been too great and the disturbing forces are reduced or eliminated. Controlling our manipulations of biogeochemical cycles so that ecosystems can continue to provide the goods and services upon which humanity depends is one of the major challenges facing modern societies.



Since 1972, the United States and Canada have invested more than 8 billion dollars to improve municipal waste facilities and reduce discharges of phosphorus into Lake Erie.

Chapter Summary

► The organisms living in a particular area, together with the physical environment with which they interact, constitute an ecosystem. At a global scale, Earth is a single ecosystem.

Climates on Earth

- Air and water movements on Earth are driven primarily by solar radiation.
- Climates determine the amount of heat, moisture, and sunlight available to living organisms in different places on Earth.
- Rising air expands and cools, releasing moisture. Descending air warms and dries and takes up moisture, creating rain shadows. Review Figure 56.1
- Global air circulation is driven by solar radiation and the spinning of Earth on its axis. Review Figure 56.2

1006 CHAPTER FIFTY-SIX

- Oceanic currents are driven primarily by prevailing winds. Review Figure 56.3

Energy Flow through Ecosystems

- The capture of solar radiation by photosynthesis powers ecosystem productivity.
- The annual production of an area is determined primarily by temperature and moisture. Review Figures 56.4, 56.5
- Energy flows through ecosystems as organisms capture and store energy and transfer it to other organisms when they are eaten. Organisms are grouped into trophic levels according to the number of steps through which energy passes to get to them. Review Table 56.1
- Who eats whom in an ecosystem can be diagrammed as a food web. Review Figure 56.6
- The amount of energy flowing through an ecosystem depends on net primary production and on the efficiency of transfer of energy from one trophic level to another. Review Figures 56.7, 56.8
- A few deep-sea and cave ecosystems are powered by chemosynthesis rather than photosynthesis.
- Through agriculture, humans manipulate ecosystem productivity so as to increase the yield of products useful to us. In modern agriculture, the energy required to do this is provided by fossil fuels.

Cycles of Materials through Ecosystem Compartments

- The main compartments of the global ecosystem are the oceans, fresh waters, land, and atmosphere, among which materials are constantly being exchanged.
- Primary production in the oceans is highest adjacent to continents in zones of upwelling, where nutrient-rich waters rise to the surface. Review Figure 56.10
- Temperate-zone lakes turn over twice each year as water cools and warms. Review Figure 56.11

Biogeochemical Cycles

- The elements organisms need in large quantities cycle through organisms to the environment and back again.
- The hydrological cycle is driven by evaporation of water, most of it from ocean surfaces. Review Figure 56.12

* ■ Atmospheric carbon dioxide is the immediate source of carbon for terrestrial organisms, but only a small part of Earth's carbon is found in the atmosphere. Review Figure 56.13

^ Increasing concentrations of carbon dioxide in the atmosphere are changing climates and influencing ecological processes. Review Figures 56.14, 56.15

- Although nitrogen makes up 78 percent of Earth's atmosphere, nitrogen can be converted into biologically useful forms only by a few species of bacteria and cyanobacteria. Review Figure 56.16
- Acid precipitation, an important regional consequence of human modifications of the nitrogen and sulfur cycles, is being addressed in the United States by flexible regulations. Review Figure 56.17

- The phosphorus cycle differs from the cycles of carbon and nitrogen in that it lacks a gaseous phase.
- The most striking effect of altering the phosphorus cycle is lake eutrophication.

For Discussion

1. Continental climates are largely confined to the Northern Hemisphere. Why are there so few continental climates in the Southern Hemisphere?
2. The amount of energy flowing through a food chain declines more or less rapidly depending upon the nature of the organisms in the chain. Which of the following simplified food chains is likely to be more efficient? Why? What criteria of efficiency are you using?
 - a. phytoplankton → zooplankton → herring
 - b. shrubs → moose → wolf
3. What principles of ecosystem functioning underlie modern agricultural practices? Which components of those practices are most likely to be unsustainable in the long term?
4. How would you expect temperature and oxygen profiles to appear in a broad, shallow tropical lake? In a very deep tropical lake? A subarctic lake? Why?
5. The waters of Lake Washington, adjacent to the city of Seattle, rapidly returned to their preindustrial condition when sewage was diverted from the lake to Puget Sound, an arm of the Pacific Ocean. Would all lakes being polluted with sewage clean themselves up as rapidly as Lake Washington if pollutant input were stopped? What characteristics of a lake are most important to its rate of recovery following removal of pollutant inputs? What are the likely ecological effects of the diverted sewage in Puget Sound?

Tropical forests currently are being cut at a very rapid rate. Does this necessarily mean that deforestation is a major source of input of carbon dioxide to the atmosphere? If not, why not?

Why do elevated atmospheric concentrations of carbon dioxide have direct effects on plants but not on animals? What are the most important indirect effects on animals, and by what ecological pathways do they operate?

The two drawings below represent pyramids of biomass for (a) an old field in Georgia and (b) the English Channel. Explain the significance of the inversion of the second pyramid compared with the first.

Carnivores Herbivores

6.

9.

(a)

Green plants

Carnivores

Zooplankton

(b)

Phytoplankton

A government official authorizes the construction of a large power plant in a former wilderness area. Its smokestacks discharge great quantities of waste resulting from the combustion of coal. List and describe all likely ecological results at local, regional, and global levels. Now suppose the wastes were thoroughly scrubbed from the stack gases. Which of the ecological results you have just outlined would still happen?

57

Biogeography

When the first Europeans arrived in Australia, they saw plants and animals that differed

in perplexing ways from the ones they knew at home. Among the oddities they found were flowers pollinated by brush-tongued parrots and mammals that hopped around on their hind legs, carrying their offspring in pouches. The first Europeans to visit North America felt more at home because the plants and animals of North America were more similar to those of Europe.

During their worldwide travels, European explorers found many vegetation types—tropical forests, mangrove forests, and deserts with tall cacti—that were unfamiliar to them, but they also found many areas where the vegetation forms and species were similar to what they knew back home. The study of the distribution of organisms over Earth's surface began when those eighteenth-century travelers first noted intercontinental differences in biotas and attempted to understand them.

Biogeography is the science that attempts to explain patterns of variation among individuals, populations, species, and communities across Earth. In this chapter, we will show how biogeographers study both events in the remote past and current ecological processes to discover how they influence the distribution patterns we see today.

Why Are Species Found Where They Are?

Explaining species' distributions might seem to be a simple matter because the question of why a species is or is not found in a certain location has only a few possible answers:

- If a species occupies a particular area, either it evolved there, or it evolved elsewhere and dispersed to that area.
- If a species is not found in a particular area, either it evolved elsewhere and never dispersed to that area, or it was once present in that area but no longer lives there.

Unfortunately, determining which of these answers is correct turns out to be far from simple.

To explain the distributions of organisms, biogeographers must draw upon and interpret a broad array of know-

Strange Organisms in New Places

Kangaroos that hopped on large hind legs and carried their offspring in pouches were novel sights to the Europeans who colonized Australia.

Answering the questions listed above requires information about the evolutionary histories of species, which comes from fossils and from knowledge of their phylogenetic relationships. It also requires information about changes in Earth itself—continental drift, glacial advances and retreats, sea level changes, and mountain building—during the time the organisms were evolving. Such geological information can tell us whether organisms evolved where they are currently found or dispersed to colonize new areas from a distant area of origin. In this section we show how the acceptance of continental drift and the use of new methods of reconstructing phylogenies changed the science of biogeography:



1008 CHAPTER FIFTY-SEVEN

Ancient events influence current distributions

Early biogeographers believed in an unchanging Earth that was too young to account for the diversity and distribution of life by any means except divine creation. Linnaeus, for example, believed that all organisms had been created in one place—which he called Paradise—from which they later dispersed. Indeed, because most people believed that the continents were fixed in their positions, the only way to account for current distributions was to invoke massive dispersal.

The notion that the continents might have moved was not seriously considered until 1912. Alfred Wegener, the German meteorologist who proposed the idea of continental drift, based his theory on several observations:

- the shapes of continents (the outlines of Africa and South America seemed to fit together like pieces of a puzzle)
- the alignment of mountain chains, rock strata, coal beds, and glacial deposits on different continents
- the distributions of organisms (the distributions of species in Africa and South America were hard to explain if one

assumed that the continents had never moved)

When Wegener proposed his ideas, few scientists took them seriously, primarily because there were no known mechanisms to move continents and because no convincing geological evidence of such movements existed. As we learned in Chapter 20, geological evidence and plausible mechanisms were eventually discovered, and the broad pattern of continental movement is now clear.

About 280 million years ago—the continents were united

to form a single land mass, Pangaea (see Figure 20.13). By the early Mesozoic era (about 245 million years ago), when the continents were still very close to one another, many groups of organisms in each of the

warm tropical regions and various polar regions

evolved. The ancestors of some organisms that live on widely separated continents today were probably present on those land masses when they were part of Pangaea.

By 100 million years ago, Pangaea had separated into northern (Laurasia) land masses, and the southern continents were drifting away from each other (see Figure 20.15). Eventually, continental drift, which continues today, brought India from Africa to southern Asia, Australia closer to Southeast Asia, and South America, which had drifted as an island for 60 million years, into contact with North America. Continental drift has thus influenced the evolution and mixing of species throughout the history of life on Earth.

Modern biogeographic methods

As the great age of Earth and the fact of evolution began to be understood, two groups of investigators developed new methods for generating testable hypotheses about geographic distribution. One group of biogeographers

current distributions are influenced by interactions

among species and by interactions between species and their physical environments. They examine species interactions of the types discussed in Chapter 55 to explain patterns of local and regional species diversity. We will see

some examples of their work later in this chapter

The other group of investigators consists of historical biogeographers who concentrate on longer time frames and larger spatial scales. They ask questions such as

- Where and when did evolutionary lineages originate?
- How did they spread?
- What do the present-day distributions of organisms tell us about their past histories?

An important technique developed by historical biogeographers was the transformation of taxonomic phylogenies into "area phylogenies" by substituting the taxa's geographic distributions for their names. Distribution patterns identified in this manner may suggest routes of dispersal or point to the splitting of biotas due to the appearance of barriers to dispersal. For example, by combining the phylogenetic relationships and current distribution pattern of the horse family, we can better understand why its members are found where they are and where past barriers probably influenced speciation events among them (Figure 57.1).

If we compare the distribution patterns of many evolutionary lineages, we may detect similarities and differences. Similarities suggest common responses to physical events, such as continental drift, mountain building, and sea-level changes. Differences suggest that organisms in different lineages responded in different ways or at different times, or that they dispersed in different ways and at different times.

The Role of History in Biogeography

Past events influence today's patterns of distribution. We can never know past events with complete certainty, but by using a variety of types of evidence, historical biogeographers can develop and test hypotheses in which they eventually have a high degree of confidence. As we have just seen, biogeographers often base their interpretations on phylogenies, which show the evolutionary relationships among the organisms in a lineage (see Chapter 23). Phylogenies are most useful to biogeographers if the approximate times of evolutionary and geographic separations of lineages can be estimated.

Biogeographers use several approaches to infer the approximate times of separation of taxa within a lineage. First, if a taxon has been ticking at a relatively constant rate, the degree of difference in the molecules of species will be strongly correlated with time their lineages have been evolving independently (see Chapter 24). Second, fossils can help to show how long a taxon has been present in an area and whether its members formerly lived in areas where they are no longer found. The fossil record is helpful, but it is always incomplete. The first and



1009

Phylogeny

Onager

Ancestral horse

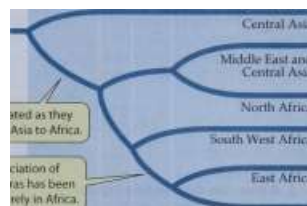


'Area phylogeny'

Origin in Asia

Mountain zebra

Plains zebra



Horses speciated as they moved from Asia to Africa.

Speciation of zebras has been entirely in Africa.

East Africa

Eastern and Southern Africa

57.1 Phylogeny to "Area Phylogeny"

Conversion of a phylogeny to an "area phylogeny" helps explain how current distributions of horses came about.

3.9

3 2 1

Millions of years ago (mya)

last members of a taxon to live in an area are extremely unlikely to have become fossils that are discovered and described. Therefore, dates of colonization and extinction cannot be estimated accurately. A third valuable source of information is the distributions of living species. Much more complete and extensive information can be gathered on current distributions than will ever be available from fossils. Much can be learned by examining the distribution patterns of many different groups of living organisms. Similarities in their distributions provide clues about past events that affected many of them.

Vicariance and dispersal can both explain distributions

As we have seen, a species may be found in an area either because it evolved there or because it dispersed to that area. But what if a species is found in two or more different places? What accounts for such a split distribution? There are two possibilities.

► A barrier may appear that splits a species' distribution. The barrier may be a physical barrier and dispersal need be postulated to account for a split distribution.

► Members of a species may cross an already existing barrier and establish a new population. In this case, the species' split range must be attributed to dispersal.

By studying a single evolutionary lineage, a biogeographer may discover evidence suggesting that the distributions of its ancestors were influenced by a vicariant event, such as a change in sea level, mountain building, or continental movement. If that inference is correct, species in other lineages are likely to have been influenced by the same event and should therefore have similar distribution patterns.

Differences in distribution patterns among lineages indicate either that the lineages responded differently to the same vicariant events, that they separated at different times, or that they have very different dispersal abilities. By analyzing such similarities and differences among lineages, biogeographers can discover the relative roles of vicariant events and dispersal in determining today's distribution patterns.

Species, genera, and families found in only one place are said to be endemic to that location. As far as we know—all species are endemic to Earth. Some species are endemic to one continent. Others are restricted to very small areas, such as tiny islands or single mountaintops. Because a species may disperse widely and then die out where it originated,

1010 CHAPTER FIFTY-SEVEN

North Island



Cook

Strait

South Island

Pliocene ^X\ geography P X-^,

. o(>t



Prior to modern times, Cook Strait separated South Island and this peninsula, which then became part of North Island.

57.2 A Vicariant Distribution Explained

Blue circles indicate the current distribution of the weevil *Lyperobius huttoni*. Compare New Zealand's present geography with that of the Pliocene (inset), when the southern part of today's North Island was part of South Island.

quires the smallest number of unobserved events to a c-cojintior-itr-To see the application of the parsimony principle to biogeography, consider the distribution of the New Zealand flightless weevil *Lyperobius huttoni*, a species that is found in the mountains of South Island and on sea cliffs at the extreme southwestern corner of North Island (Figure 57.2). If you knew only its current distribution and the current positions of the two islands, you might guess that, even though this weevil cannot fly, *L. huttoni* had somehow managed to cross Cook Strait, the 25-km body of water that separates the two islands.

However, more than 60 other animal and plant species, including other species of flightless insects, live on both sides of Cook Strait. It is unlikely that all of these species made the same ocean crossing. In fact, that assumption is unnecessary to explain the distribution patterns. Geological evidence indicates that the present-day southwestern tip of North Island was formerly united with South Island. Therefore, none of the 60 species need have made a water crossing. A single vicariant event—the separation of the northern tip of South Island from the remainder of the Island by the newly formed Cook Strait—could have split aU of the

distributions. Although organisms do cross oceanic and terrestrial barriers, biogeographers often apply the parsimony principle when interpreting distribution patterns, just as evolutionists do when reconstructing phylogenies.

biogeographers cannot assume that a species now endemic to a location originated there. Endemic taxa can be very old ones that are in the process of becoming extinct, or very young taxa that have recently evolved in a restricted area.

The longer an area has been isolated from the rest of the world,

by a vicariant event, the more endemic taxa it is likely to

have, because there has been more time for evolution to take place.

Australia, which has been separated the longest from the other continents (about 65 million years), has the most distinct biota. South America has the next most distinct biota, having been isolated from other continents for nearly 60 million years. North America and Eurasia, which were joined together for much of Earth's history, have very similar biotas. That is why the early European travelers felt more at home in North America than in Australia.

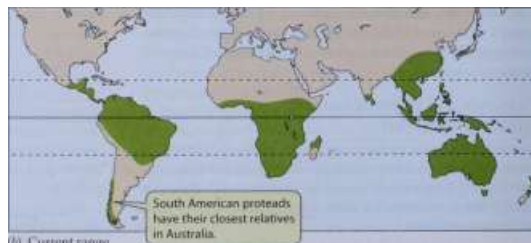


Biogeographers use parsimony to explain distributions

When several hypotheses can explain a pattern, scientists typically prefer the most parsimonious one—the one that requires the fewest evolutionary changes.

(a) *Lencospermum conocarpodendron*

Banksia integrifolia



(b) Current range

57.3 Protead Distributions Reveal a Gondwana Ancestry

(a) Proteads from South Africa (left) and Australia (right).

(b) Current distribution (green) of the family Proteaceae.

Biogeographic histories are reconstructed from various kinds of evidence

Biogeographers use distribution maps, phylogenies, and knowledge of ancient climates and ancient geography to reconstruct the biogeographic histories of taxa. These kinds of information suggest, for example, that distributions of the proteads (family Proteaceae; Figure 57.3) have been influenced by continental drift many millions of years ago. These plants are found in Africa, but the African species are highly specialized members of an endemic subfamily. The South American species are members of a different subfamily, whose closest relatives are found in the Australian region. The phylogeny and distribution of proteads suggests that they had a broad distribution in Gondwana before that large continent began to break up, and that populations were carried by drifting land masses to their current locations. Protead lineages have been separated on the different continents long enough to have evolved major differences from one another.

Earth can be divided into biogeographic regions

Although the drifting continents carried many kinds of organisms with them, they have been isolated from one another long enough for distinct biotas to have evolved on them. Differences among continental biotas form the basis for dividing Earth into six major biogeographic regions: the Nearctic, Neotropical, Palearctic, Ethiopian, Australian, and Antarctic regions. Biogeographers drew the boundaries of these regions where species compositions change

dramatically over short distances (Figure 57.4). These biogeographic regions are based on taxonomic similarities among the organisms living in them, not on their appearances, and should not be confused with the biomes we will discuss later in this chapter.

Biogeographers agree on the boundaries of many of these regions, but plant biogeographers recognize two regions not used by zoogeographers: southern South America and the Cape Region of South Africa. The floras of these two regions are distinct from those of adjacent areas on those continents, but the faunas of southern South America and the Cape Region are very similar to those of the remainder of those continents.

Except for the Australasian region, the biogeographic regions are no longer separated from each other by water as they were in the past. The biological distinctness of these biogeographic regions is maintained today in part by mountain and desert barriers to dispersal and in part by major climatic changes over short distances.

Ecology and Biogeography

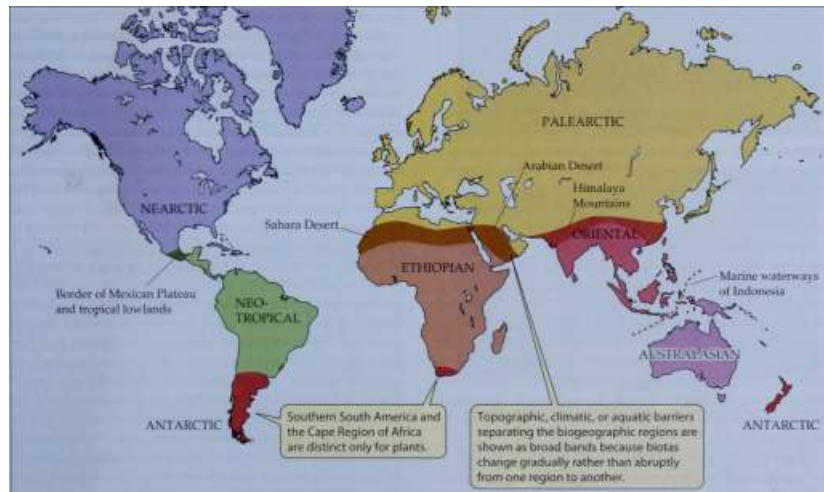
Ecological biogeographers use the wealth of available information on current distributions of organisms to test theories that explain the numbers of species in different com-

-&s*

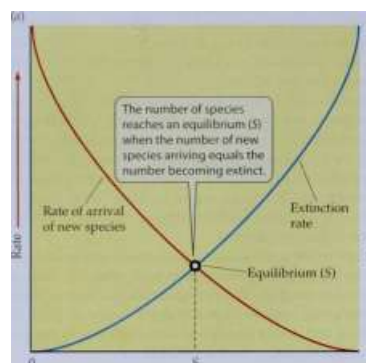
q

57.4 Major Biogeographic Regions

The biotas of Earth's major biogeographic regions differ strikingly from one another.



1012 CHAPTER FIFTY-SEVEN



Island near mainland

Small island

Number of species

57.5 A Model of Species Richness on Islands

Rates of arrival of new species and extinction of species already present determine the equilibrium species richness.

munities, the ways in which species disperse, and the effects of different types of barriers to dispersal. They can also use experiments to test some hypotheses. Here we describe an influential biogeographic model that attempts to account for the number of species living in an area—its species richness—and then look at experiments conducted to test this model.

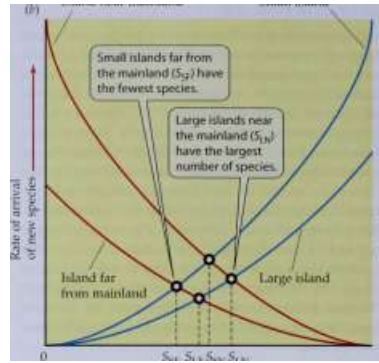
The species richness of an area is determined by rates of colonization and extinction

Over periods of a few hundred years (a time span during which speciation is unlikely), the species richness of an area depends on the immigration of new species and the extinction of species already present. It is easiest to visualize the effects of these two processes if we consider, as did Robert MacArthur and Edward Wilson, an oceanic island that initially has no species.

Imagine a newly formed oceanic island that receives colonists from a mainland area. The list of species on the mainland that might possibly colonize the island is called the species pool. The first colonists to arrive on the island are all "new" species because no species live there initially. As the number of species on the island increases, a larger fraction of colonists will be members of species already present. Therefore, even if the same number of species arrive as before, the rate of arrival of new species decreases, until it reaches zero when the island has all the species in the species pool.

Now consider extinction rates. First there will be only a few species on the island, and their populations may grow large. As

more species arrive and their populations in-



$S_{sf} S_{lf}^{sn} \ln$

Number of species

crease, the resources of the island will be divided among more species. We therefore expect the average population size of each species to become smaller as the number of species increases. The smaller a population, the more likely it is to become extinct. In addition, the number of species that can become extinct increases as species accumulate on the island. New arrivals to the island may include pathogens and predators that increase the probability of extinction of other species, further increasing the number of species becoming extinct per unit of time.

Because the rate of arrival of new species (i.e., colonization) decreases and the extinction rate increases as the number of species increases, eventually the number of species should reach an equilibrium at which the rates of arrival and extinction are equal (Figure 57.5a). If there are more species than the equilibrium number, extinctions should exceed arrivals, and species richness should decline. If there are fewer species than the equilibrium number, arrivals should exceed extinctions, and species richness should increase. The equilibrium is dynamic because if either rate fluctuates—as they generally do—the expected number of species shifts up and down.

Even if extinction and colonization rates are assumed to fluctuate somewhat, the model can be used to predict how species richness should differ among islands of different sizes and different distances from the mainland species pool. We expect extinction rates to be higher on small islands than on large islands because species' populations will, on average, be smaller there. Similarly, we expect fewer colonists to reach islands more distant from the mainland. Figure 57.5b gives hypothetical relative species richnesses for islands of different sizes and distances from the mainland. As you can see, the number of species should be highest for islands that are relatively large and relatively close to the mainland.

01

Z

100 -

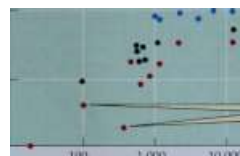
10

BIOGEOGRAPHY

1013

- More than 3,200 km from New Guinea
- 800-3,200 km from New Guinea
- Less than 800 km from New Guinea

ID



Large islands near the mainland harbor more species...

...than small islands far from the mainland.

1,000 10,000

Island size (km²)

100,000

57.6 Small, Distant Islands Have Fewer Species

The dots show the numbers of land and freshwater bird species on islands of different sizes in the Moluccas, Melanesia, Micronesia, and Polynesia. These islands have been divided into three groups according to their distance from the "mainland," which is New Guinea.

The island biogeographic model has been tested

The island biogeographic model we have just described predicts that the number of species should increase with island size and decrease with distance from the mainland. Ecological biogeographers have tested the model using counts of species on real islands. New Guinea, which is large enough to function as a small continent, supplies the mainland species pool for islands farther east in the Pacific Ocean. The number of bird species found on those islands corresponds to the predictions of the model (Figure 57.6). The species richness patterns of plants, insects, lizards, and mammals on those islands are similar to those of birds.

The predictions of the island biogeographic model are based on assumptions about rates of colonization and extinction. Major disturbances, which serve as "natural experiments," sometimes permit colonization and extinction rates to be estimated directly. The eruption of Krakatau in August 1883, described in Chapter 20, destroyed all life on the island's surface. After the lava cooled, Krakatau was colonized rapidly by plants and animals from Sumatra to the east and Java to the west.

57.1

Number of Species of Resident Land Birds on Krakatau

PERIOD

NUMBER OF SPECIES

EXTINCTIONS COLONIZATIONS

17 4

7 7

During the 1920s, a forest canopy was developing, and there were high rates of colonization by both birds (Table 57.1) and plants. Birds probably brought the seeds of many plants because, between 1908 and 1934, both the percentage (from 20 to 25 percent) and the absolute number (from 21 to 54 species) of plant species with bird-dispersed seeds increased. By 1933, Krakatau was again covered with a tropical evergreen forest, and 271 species of plants and 29 species of resident land birds were found there. Today the numbers of species of plants and birds are not increasing as fast as they did during the 1920s, but there are still colonizations and extinctions, as predicted by the model.

A manipulative experiment to test the island biogeographic model was carried out in the Florida Keys, a region dotted with thousands of small islands consisting entirely of red mangrove trees rooted in shallow water. Six tiny islands were fumigated with methyl bromide, which destroyed all arthropods living on them (Figure 57.7). Methyl bromide decomposes rapidly and does not inhibit recolonization.

The design of this experiment permitted the investigators to measure colonization and extinction rates directly.

RESEARCH METHOD

|

Scaffolding was erected to enclose a small mangrove island in the Florida Keys.

• v - ' - :

-

. II



Methyl bromide introduced into the enclosure killed all arthropods inside it.

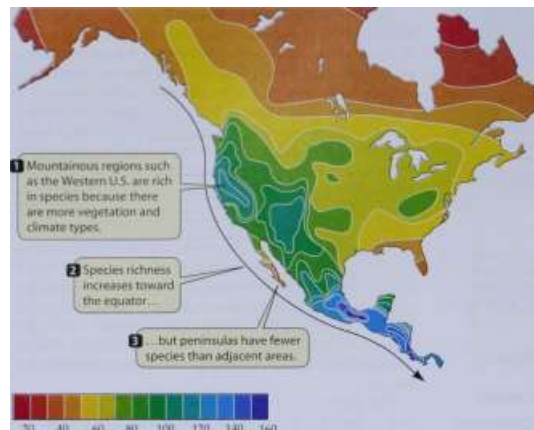


Over about a year, investigator observed and measured the rates of recolonization.

57.7 Experimental Island Biogeography

Scientists removed all species of arthropods from several small mangrove islands in the Florida Keys by enclosing them and fumigating them with methyl bromide. When the enclosures were removed, arthropods quickly recolonized the islands.

1014 CHAPTER FIFTY-SEVEN



40 60 80 100 120 140 160 Number of mammal species

They found that rates of colonization of the islands by arthropods were very high. Within a year the fumigated islands had about their original number of species, and each census revealed considerable turnover in the number of species present. Both results support the island biogeographic model.

Species richness varies latitudinally

A nearly universal pattern in the distribution of species is that more species live in tropical than in high-latitude regions. Figure 57.3 shows the latitudinal gradient in mammal species richness in North and Central America. Similar patterns exist for birds, frogs, and trees, and for many marine taxa.

The figure also shows two other general patterns of species richness. First, more species are found in mountainous regions than in relatively flat areas because more vegetation types and climates exist within topographically complex regions. Second, species richness declines on peninsulas, such as Florida and Baja California, probably because colonization is possible from only one direction.

®

Terrestrial Biomes

Another way in which ecologists describe the distribution patterns of organisms is by classifying ecosystems. They apply the name biome to a major ecosystem type that differs from other types in the structure of its dominant vegetation. The vegetation of a biome has a similar appearance wherever on Earth that biome is found, but the plant species in these communities, despite their physical similarity,

57.8 Latitudinal Gradient of Species Richness of North American Mammals

Lines on the figure connect regions with equal numbers of species. An increase in species richness toward the equator typifies many other taxa, such as birds, amphibians, and trees, as well as mammals.

ities, may not be evolutionarily closely related. Although biomes are named for and identified by their characteristic plants, sometimes supplemented by their location or climate, each biome contains many other kinds of organisms. The geographic distribution of biomes is shown in Figure 57.9.

Biomes are identified by their distinctive climates and dominant plants

Because climate plays a key role in determining which types of plants live in a given place, the distribution of biomes on Earth is strongly influenced by annual patterns of temperature and rainfall. In some biomes, such as temperate deciduous forest, precipitation is relatively constant throughout the year, but temperature varies strikingly between summer and winter. In other biomes, both temperature and precipitation change seasonally. In certain biomes, such as tropical rainforest, temperatures are nearly constant, but rainfall varies seasonally.

In the tropics, where seasonal temperature fluctuations are small, annual climatic cycles are dominated by wet and dry seasons. In general, the number of months during which a region is close to the intertropical convergence zone (and hence receives rainfall) increases toward the equator (see Chapter 56). The intertropical convergence zone shifts latitudinally in a seasonally predictable way, resulting in a characteristic latitudinal pattern of distribution of rainy and dry seasons in tropical and subtropical regions (Figure 57.10).

Pictures and graphs capture the essence of terrestrial biomes

It is easiest to grasp the similarities and differences among terrestrial biomes by means of a combination of photographs and graphs of temperature, precipitation, and biological activity, supplemented by a brief description of the species richness and other attributes of those biomes. We use this method in the following pages to describe the major terrestrial biomes of the world.

► Each biome is represented by two photographs that show either the biome at different times of year or representatives of the biome in different places on Earth.



Tropical evergreen forest

Tropical deciduous forest

Tropical thorn forest

Savanna

Hot desert

Chaparral

High mountains (boreal forest and tundra) Temperate evergreen forest Temperate deciduous forest Boreal forest

1 I Cold desert

] Arctic tundra

| Temperate grassland

~] Polar ice cap

23°N -

57.9 Biomes Have Distinct Geographic Distributions

Compare these biomes with the patterns of net primary production shown in Figure 56.5.

► The first set of graphs plots seasonal patterns of temperature and precipitation at a typical site in the biome.

► Other graphs show how active different kinds of organisms are during the year. Levels of biological activity (shown by the width of horizontal bars) change either because resident organisms become more active (produce leaves, come out of hibernation, hatch, or reproduce) or because organisms migrate into and out of the biome. (The patterns shown are for the Northern Hemisphere; for high-latitude biomes, patterns in the Southern Hemisphere are 6 months out of phase with those illustrated.)

► A small box describes the dominant growth forms of plants in the biome and patterns of species richness there.

These descriptions are very general; they cannot capture the variation that exists within each biome.

23°S -

Dry season



57. 7 0 Rainy and Dry Seasons Change with Latitude

In the tropics and subtropics, which months are rainy and which are dry is highly predictable based on the region's latitude.

1016 CHAPTER FIFTY-SEVEN

TUNDRA

20°C is a

"comfortable" 68°F.

Temperature

0 C is the freezing point of water

(=32 C F).

(

15

10 h 5 ^0

-5 -10 h -15 -20 -25

Upernavik, Greenland 73 N

Winter is very cold and long.

Summer is cool and short.

T

Range 28°C

Jan

Jul

Dec

i n cm

5 cm equals [just over 2 inches. J o

Biological Activity

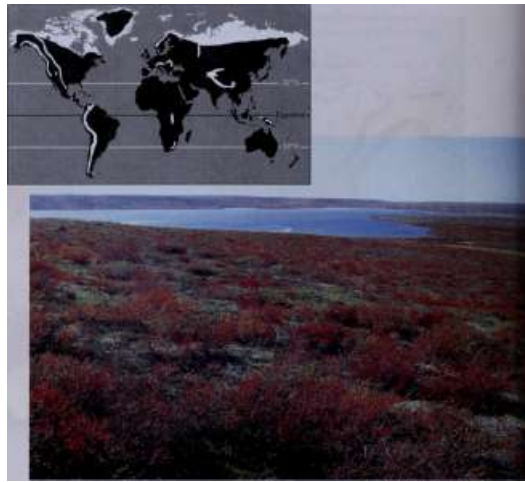
Photosynthesis

Flowering

_Zi

Fruiting

Mammals



Arctic tundra: Northwest Territories, Canada



Jan Jul

Community Composition

Dec



Tropical alpine tundra: Teleki Valley, Mt. Kenya

Tundra is found at high latitudes and in high mountains

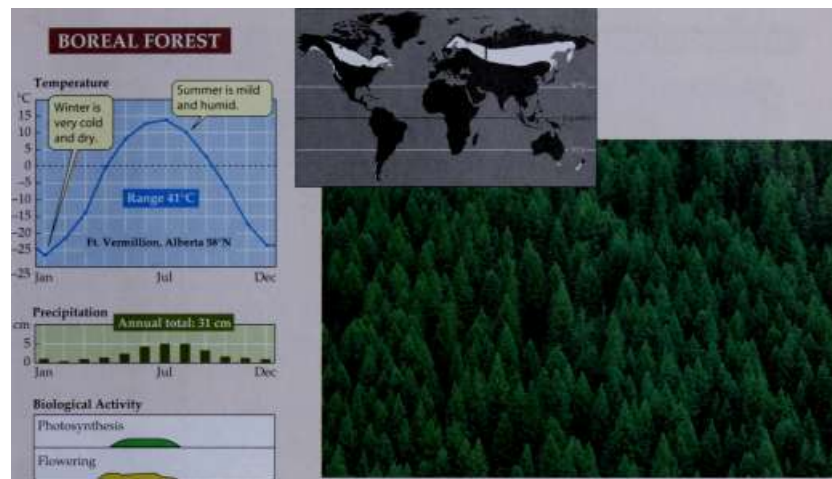
The tundra biome is found at high latitudes in the Arctic, Antarctic, and high in mountains at all latitudes (where it is called alpine tundra). There are no trees; the vegetation is dominated by short perennial plants.

Permanently frozen soil—permafrost—underlies tundra

vegetation. The top few centimeters of soil thaw during the short summers, when the sun shines 24 hours a day. Even though there is little precipitation, Arctic tundra is very wet because water cannot drain down through the frozen soil. Plants grow for only a few months each year. Most Arctic tundra animals either migrate into the area only for the summer or are dormant for most of the year.

BIOGEOGRAPHY 1017

BOREAL FOREST



Fruiting

Mammals



Soil Biota

Northern conifer forest, Gunnison National Forest, Colorado

an Jul

Community Composition

Dec



Bryophytes and lichens on southern evergreens, Tasmania, southern Australia

Boreal forests are dominated by evergreen trees

The boreal forest biome is found equatorward from, or at lower elevations on temperate-zone mountains than, tundra. Winters are long and very cold, and summers are short (although often warm). The short summers favor trees with evergreen leaves because these trees are ready to photosynthesize as soon as temperatures warm in spring.

The boreal forests of the Northern Hemisphere are dominated by coniferous evergreen gymnosperms. In the

Southern Hemisphere, the dominant trees are southern beeches (*Nothofagus*). Evergreen forests also grow along the west coasts of continents at middle to high latitudes, where winters are mild but very wet and summers are cool and dry. These forests are home to Earth's tallest trees.

Boreal forests have only a few tree species. The dominant animals—such as insects, moose, and hares—eat leaves. The seeds in the cones of conifers also support a fauna of rodents and birds.

1018 CHAPTER FIFTY-SEVEN

TEMPERATE DECIDUOUS FOREST



A Rhode Island forest in summer and...

in winter

Temperature



Winter is cold and snowy.

Summer is warm and moist.

Range 31°C

Madison, Wisconsin 43 = N

Jan Precipitation

Jul

Dec

10 5 0

Annual total: 81 cm

111

an

■ Ulli.-l

Jul Dec

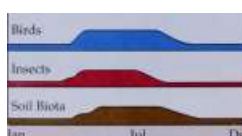
Biological Activity



Photosynthesis

Flowerin

Mammals



Community Composition

Dominant Plants

Trees and shrubs

Species Richness

Plants: Many tree species in Southeast USA and East Asia, rich shrub layer Animals: Rich; many migrant birds, richest amphibian communities on Earth, rich summer insect fauna

Rich

Temperate deciduous forests change with the seasons

The temperate deciduous forest biome is found in eastern North America, eastern Asia, and western Europe. Temperatures in these regions fluctuate dramatically between summer and winter. Precipitation is relatively evenly distributed throughout the year. Deciduous trees, which dominate these forests, produce leaves that photosynthesize rapidly

during the warm, moist summers and lose their leaves during the cold winters.

There are many more tree species here than in boreal forests. The temperate forests richest in species are in the southern Appalachian Mountains of the United States and in eastern China and Japan, areas that were not disturbed by Pleistocene glaciers. Many birds migrate into this biome in summer, when insects are abundant.

BIOGEOGRAPHY 1019

TEMPERATE GRASSLANDS

°C

30

25 20 15 10

5 0

cm

Temperature

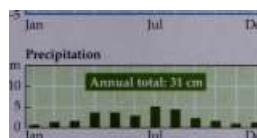
Summer is warm and wetter.

Winter is cold and dry.

T

Range 24°C

Pueblo, Colorado 38°N-



Biological Activity

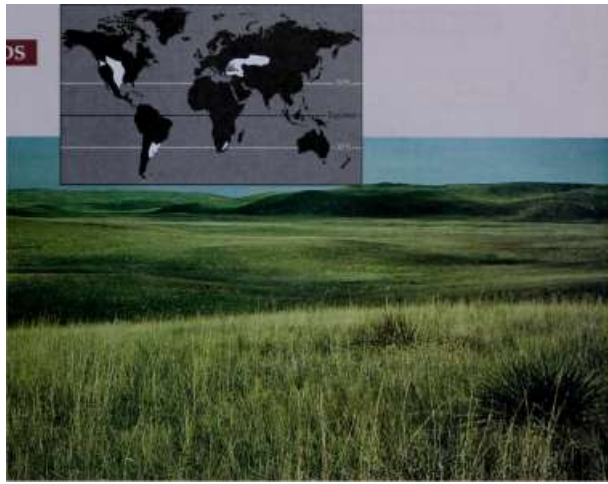


Photosynthesis

Mammals



Soil Biota



mBKbrnmamm

Nebraska prairie in spring

Tan Jul

Community Composition

Dec



!■

The Veldt, Natal, South Africa

Temperate grasslands are widespread

The temperate grassland biome is found in many parts of the world, all of which are relatively dry much of the year. Most grasslands have hot summers and relatively cold winters. In some grasslands most of the precipitation falls in winter; in others the majority falls in summer. Such regions as the pampas of Argentina, the veldt of South Africa, and the Great Plains of the United States are components of the

temperate grassland biome. Most of this biome has been converted to agriculture.

Grasslands are structurally simple, but they are rich in species of perennial grasses, sedges, and forbs. Grasses are well adapted to grazing and fire because they store much of their energy underground and quickly resprout after they are burned or grazed. As we saw in Chapter 56, many grasslands support large populations of grazing mammals.

1020 CHAPTER FIFTY-SEVEN

COLD DESERT

Te mperature

C 30

25 20 15

10 5

0

-5 -10

Winters are cold and very dry.

Summers are much warmer, but still dry.

~7-

Range 23°C

Cheyenne, Wyoming 41° N

Jan

Jul

Dec

Jan

Biological Activity

Jul

Dec

Photosynthesis

Flowering

Fruiting

Mammals

Birds

Insects

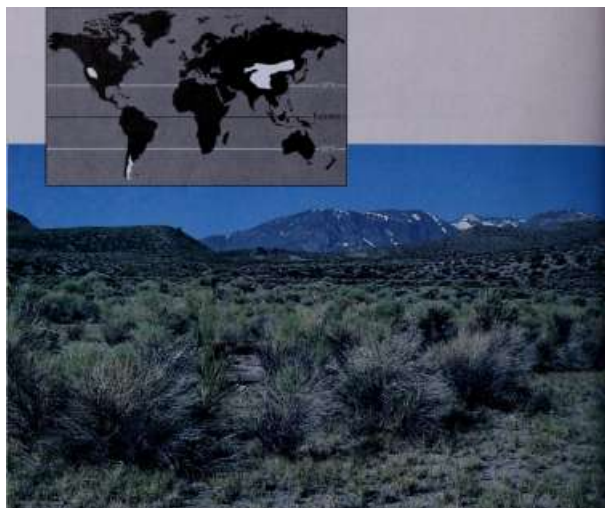
Soil Biota

Jan

Jul

Dec

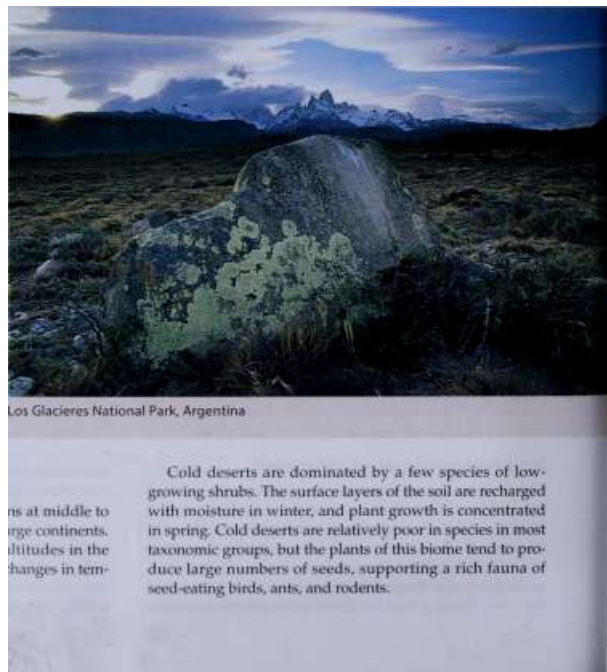
Community Composition



Sagebrush steppe near Mono Lake, California

Cold deserts are high and dry

The cold desert biome is found in dry regions at middle to high latitudes, especially in the interiors of large continents. Cold deserts also are found at fairly high altitudes in the rain shadows of mountain ranges. Seasonal changes in temperature are great.



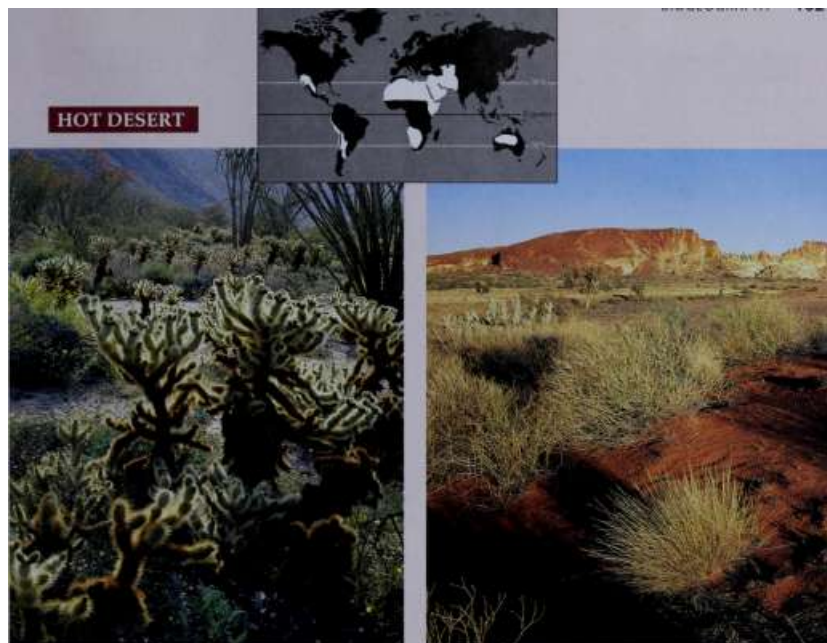
Los Glaciers National Park, Argentina

Cold deserts are dominated by a few species of low-growing shrubs. The surface layers of the soil are recharged with moisture in winter, and plant growth is concentrated in spring. Cold deserts are relatively poor in species in most taxonomic groups, but the plants of this biome tend to produce large numbers of seeds, supporting a rich fauna of seed-eating birds, ants, and rodents.

Los Glaciers National Park, Argentina

Cold deserts are dominated by a few species of low-growing shrubs. The surface layers of the soil are recharged with moisture in winter, and plant growth is concentrated in spring. Cold deserts are relatively poor in species in most taxonomic groups, but the plants of this biome tend to produce large numbers of seeds, supporting a rich fauna of seed-eating birds, ants, and rodents.

BIOGEOGRAPHY 1021



Anzo Borrego Desert, California

Rainbow Valley in the desert of central Australia

°C 40 30|-20 10|-0

Temperature

Range 29.5 C C

Khartoum, Sudan 15.5°N

N~J Winter is very warm and dry.

1

Summer is very warm.

Jan Precipitation

Jul

cm

5 0

Annual total: 15 cm

JL.

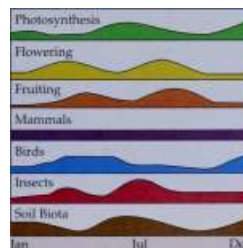
Dec

Biological Activity

Jan

Jul

Dec



Community Composition

Dominant Plants

Many different growth forms

Species Richness

Plants: Fairly high; many annuals Animals: Very rich in rodents; richest bee communities on Earth; very rich in reptiles and butterflies

Poor in species

Hot deserts form around 30° latitude

The hot desert biome is found in two belts, centered around 30°N and 30°S latitudes, respectively. These are the regions where dry air descends, warms, and picks up moisture (see Chapter 56). The driest large regions within this biome are in the center of Australia and the middle of the Sahara Desert of Africa.

Except in these driest regions, hot deserts have a richer and structurally more diverse vegetation than cold deserts. Succulent plants that store large quantities of water in their stems are conspicuous. Annual plants germinate and grow when rain falls. Pollination and seed dispersal by animals are common. Rodents and ants are often remarkably abundant, and lizards and snakes typically are rich in species and common.

1022 CHAPTER FIFTY-SEVEN

CHAPARRAL

Temperature

25 -20 -15 -10

5

0

Winter is mild and humid.

Summer is mild and very dry.

7

Range 7°C

Monterey, California 36°N

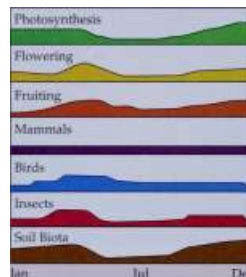
Precipitation

cm



Dec

Biological Activity



Community Composition

Dominant Plants

Low stature shrubs and herbaceous plants

Species Richness

Plants: Extremely high in South Africa and Australia

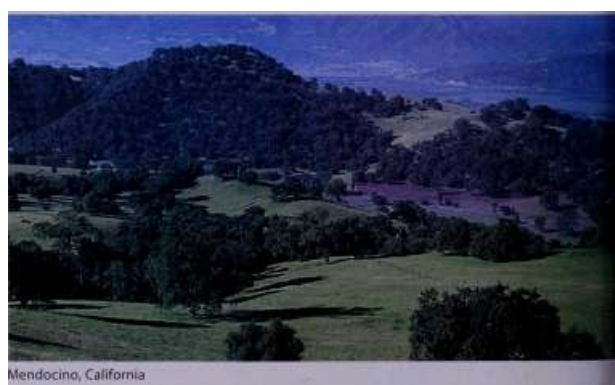
Animals: Rich in rodents and reptiles; very rich in insects; especially bees

renrfm

Moderately rich



Fynbos vegetation, Cape of Good Hope, South Africa



Mendocino, California

The chaparral climate is dry and pleasant

The chaparral biome is found on the west sides of continents at moderate latitudes, where cool ocean waters flow offshore. Winters in this biome are cool and wet; summers are hot and dry. Such climates are found in the Mediterranean region of Europe, coastal California, central Chile, extreme southern Africa, and southwestern Australia.

Chaparral is dominated by low-growing shrubs and trees that have tough, evergreen leaves. The shrubs carry out most of their growth and photosynthesis in early

spring, which is when insects are active and birds breed. Annual plants are abundant, producing seeds that are deposited in soil "seed banks." This biome thus supports large populations of small rodents, most of which store seeds in underground burrows.

Chaparral vegetation is naturally adapted to survive periodic fires. Many shrubs of Northern Hemisphere chaparral produce bird-dispersed fruits that ripen in the late fall, when large numbers of migrant birds arrive from the north.

BIOGEOGRAPHY 1023

THORN FOREST and TROPICAL SAVANNA

Temperature

°C (—{ Winters are mild and very dry. 35



Summers are very wet, but not much warmer than winter.

Range 10.7 C

cm

20

15

10

5

0

Jan Jul

Precipitation

Dec

Annual total: 74 cm

—|



an

Jul

Dec

Biological Activity

Photosynthesis

A

Flowering

Fruiting

Mammals



Thorn forest in Madagascar

Soil Biota

an

Jul

Dec

Community Composition

Dominant Plants

Shrubs and small trees; grasses

Species Richness

Plants: Moderate in thorn forest; low in savanna

Animals: Rich mammal faunas; moderately rich in birds, reptiles, and insects

Rich



Savanna in Tanzania

Thorn forests and savannas have similar climates

Thorn forests are found on the equatorial sides of hot deserts. The climate is semiarid; little or no rain falls during the winter, but rainfall may be heavy during the summer. Thorn forests contain many plants similar to those found in hot deserts. The dominant plants are spiny shrubs and small trees. Members of the genus *Acacia* are common in thorn forests worldwide.

The dry tropical and subtropical regions of Africa, South

America, and Australia have extensive areas of savannas — expanses of grasses and grasslike plants punctuated by scattered trees. The largest savannas are found in central and eastern Africa, where this biome supports huge numbers of grazing and browsing mammals that serve as prey for many large carnivores.

Grazers and browsers maintain the savannas. If savanna vegetation is not grazed, browsed, or burned, it typically reverts to

dense thorn forest.

1024 CHAPTER FIFTY-SEVEN

TROPICAL DECIDUOUS FOREST

Temperature



an Jul

Biological Activity

Photosynthesis Flowering

Fruiting Mammals



Palo Verde National Park, Costa Rica, in the rainy season.

Birds

Insects

Soil Biota

Jan Jul

Community Composition

Dec

Dominant Plants

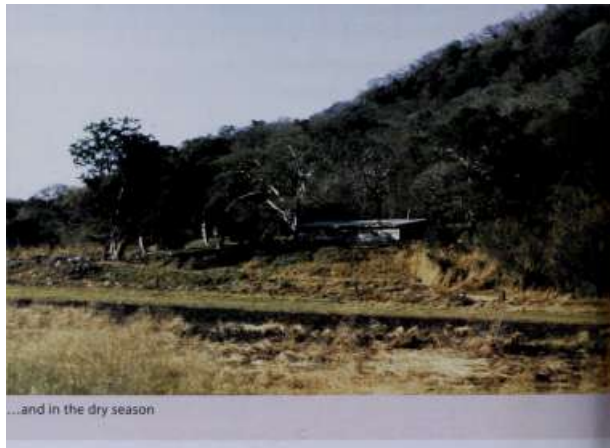
Deciduous trees

Species Richness

Plants: Moderately rich in tree species Animals: Rich mammal, bird, reptile, and amphibian communities; rich in insects

KTPI'ff*!

Rich, but poorly known



...and in the dry season

Tropical deciduous forests occur in hot lowlands

As the length of the rainy season increases toward the equator, thorn forests are replaced by tropical deciduous forests. These forests have taller trees and fewer succulent plants than thorn forests, and they are much richer in species. Most of the trees, except for those growing along rivers, lose their leaves during the long, hot dry season.

Many of them flower while they are leafless. This biome is very rich in species of both plants and animals.

The soils of the tropical deciduous forest biome are some of the best soils in the tropics for agriculture because they are less leached of nutrients than the soils of wetter areas. As a result, most tropical deciduous forests have been cleared for grazing cattle and growing crops.

BIOGEOGRAPHY 1025

TROPICAL EVERGREEN FOREST



■fc>*

*&>

3*

- -t. - ^ ^ % r r *



WKe&.-

BH HHIHIi



The exterior of lowland wet forest..
 ...and its interior, Cocha Cashu, Peru

°C 25 20 15 -10

Temperature

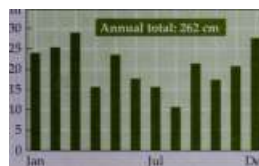
Range 2.2C

Warm and rainy all year.

Equitos, Peru 3°S

Precipitation

cm



Biological Activity

Photosynthesis Flowering

Fruiting

Mammals



Community Composition

Dominant Plants

Trees and vines

Species Richness

Plants: Extremely high Animals: Extremely high in mammals, birds, amphibians, and arthropods

Knowledge

Very rich but poorly known

Biological activity is essentially constant year round.

Tropical evergreen forests are rich in species

Tropical evergreen forests are found in equatorial regions where total rainfall exceeds 250 cm annually. This biome is the richest of all in species of both plants and animals, with up to 500 species of trees per km². Many of these species are rare. Food webs are extremely complex.

Tropical evergreen forests have the highest overall productivity of all terrestrial ecological communities. However, most mineral nutrients are tied up in the vegetation. The

soils usually cannot support long-term agriculture.

On the slopes of tropical mountains, temperature decreases about 6° for each 1,000 m of elevation. The trees are shorter than lowland tropical trees. Their leaves are smaller, and there are more epiphytes—plants that grow on other plants and derive their nutrients and moisture from air and water rather than soil. Epiphytes thrive in tropical mountain forests where clouds form, bathing the forest in moisture.

1026 CHAPTER FIFTY-SEVEN

Aquatic Biogeography

The coastal zone affected by wave action constitutes the littoral zone.

The column of water above the ocean floor constitutes the pelagic zone.

The ocean floor constitutes the benthic zone.

The abyssal and benthic zones coincide below the penetration of light.

Three-fourths of Earth's surface is covered by water, most of it in the oceans. Earth's oceans form one large, interconnected water mass with no obvious barriers to dispersal. Fresh waters, in contrast, are divided into river basins and thousands of relatively isolated lakes. For freshwater organisms that cannot survive out of water, terrestrial habitats are barriers to dispersal. However, some aquatic species have flying adults that can disperse widely among water bodies. Others have windborne, desiccation-resistant spores and seeds. Still others are small enough to be transported by means such as mud on the feet of birds. Many freshwater taxa that are capable of dispersing across terrestrial barriers are distributed widely over several continents.

Freshwater ecosystems have little water but many species

Although only about 2.5 percent of Earth's water is found in ponds, lakes, and streams, about 10 percent of all aquatic species live in freshwater habitats. Prominent among these are the more than 25,000 species of insects that have at least one aquatic stage in their life cycle. Most commonly, eggs and larvae are aquatic and adults have wings. Adults of some of these insects, such as dragonflies, are powerful flyers, but adults of mayflies and some other species are weak flyers, desiccate rapidly in air, and live no longer than a few days. As you would expect, oceanic islands have no or very few species of these weak flyers.

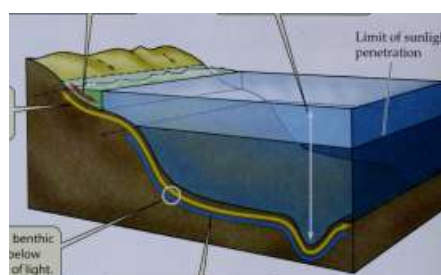
Similarly, fishes unable to live in salt water can disperse only within the connected streams and lakes of a river basin. Most families of freshwater fishes are restricted to a single continent. Those families with species distributed on both sides of major saltwater barriers are believed to be ancient lineages whose ancestors were distributed widely in Laurasia or Gondwana.

Marine biogeographic regions are determined primarily by water temperature and nutrients

Ocean water moves in great circular patterns—clockwise in the Northern Hemisphere and counterclockwise in the Southern Hemisphere (see Figure 56.3) These movements disperse organisms with limited swimming abilities. Nevertheless, most marine organisms have restricted ranges, indicating that important environmental limits to their distributions exist in the oceans.

We can divide the oceans into zones based on sharp horizontal and vertical environmental gradients (Figure 57.11). At all depths, the bottom of the ocean is called the benthic zone, and the open water column is called the pelagic zone. The ocean floor below the level of sunlight penetration is called the abyssal zone. The coastal zone from the most limits of tidal action down to the depth where

Limit of sunlight penetration



The ocean floor below the depth of sunlight penetration is also called the abyssal zone.

Zones of the ocean are shown schematically in relation to depth and sunlight penetration.

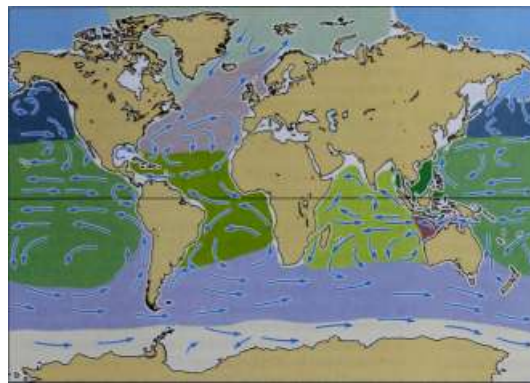
the water is thoroughly stirred by wave action is called the littoral zone.

Water temperatures, hydrostatic pressures, and food supplies all change with depth and distance from shore, influencing biotic distributions. Food is scarce, for example, in the permanently dark, cold waters of the deep sea. Living successfully in different zones of the ocean requires different physiological tolerances and morphological attributes. Not surprisingly, even though many organisms can disperse between these zones, organisms from one zone survive poorly if they attempt to live in another.

Ocean temperatures are barriers to colonization because many marine organisms are well adapted to only relatively narrow temperature ranges. The main biogeographic divisions of the pelagic zone coincide with regions where the temperature of surface waters changes relatively abruptly as a result of horizontal and vertical ocean currents (Figure 57.12). These temperature changes, in combination with seasonal changes in the amount of daylight, determine the seasons of maximum primary production. Species of marine algae tend to be adapted to photosynthesize either in summer or in winter, but not during both seasons.

Because nutrients gradually sink to the ocean bottom, high concentrations of nutrients in the pelagic zone are restricted to areas where upwelling currents bring nutrient-rich bottom waters to the surface (see Figure 56.10). Most marine organisms that grow and reproduce well in nutrient-rich waters perform relatively poorly in nutrient-poor waters. Therefore, nutrient-rich waters typically have biotas that differ considerably from those of nutrient-poor waters in the same region.

Deep ocean waters are barriers to the dispersal of marine organisms that live only in shallow water. Eggs and larvae of marine organisms can be carried great distances



Pacific polar Pacific westerly winds Pacific trade winds J Atlantic polar] Atlantic westerly winds I Atlantic trade winds

LZZ]



Indian ocean trade winds West pacific coastal Indonesian coastal Antarctic westerly winds Antarctic polar

57.12 Pelagic Regions are Determined by Ocean Currents

The arrows represent ocean currents. Regions in which photosynthesis is maximized at different seasons are indicated by different colors.

BIOGEOGRAPHY 1027

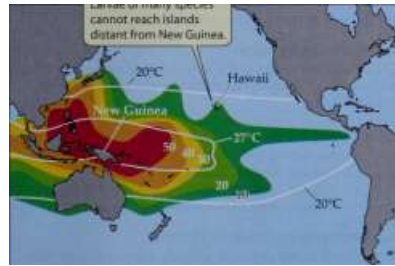
demonstrated by the richness of reef-building corals in the intertidal and sub-tidal zones of isolated islands in the Pacific Ocean, which decreases with distance from New Guinea (Figure 57.13).

Marine vicariant events influence species distributions

Ancient vicariant events associated with continental drift do not influence current distributions of marine organisms, but more recent events have left biogeographic traces. An important recent vicariant event was the formation of the Isthmus of Panama about 3 million years ago. The isthmus separated the Pacific Ocean from the Caribbean Sea for the first time in more than 100 million years. Distinct marine biotas are now evolving on opposite sides of the isthmus. It forms a barrier to the dispersal of Pacific species, such as sea snakes, which reached the west coast of the Americas after the isthmus formed (Figure 57.14). Currently the fresh waters of Gatun Lake form a barrier to the dispersal of marine organisms through the Panama Canal. If a sea-level canal were constructed across the isthmus, poisonous sea snakes and other marine organisms would be able to disperse into the Caribbean.

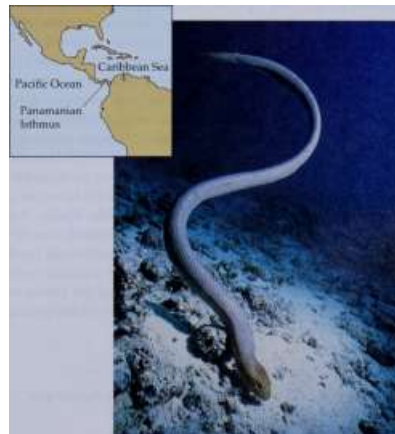
by ocean currents, but the distance they can disperse is determined in large part by the duration of the larval life span. Relatively few species have eggs and larvae that survive long enough to disperse across wide barriers of deep water and settle in new areas. The effect of these barriers is

Larvae of many species cannot reach islands distant from New Guinea.



57.13 Generic Richness of Reef-Building Corals Declines with Distance from New Guinea

The lines connect areas with equal numbers of genera. Since temperature also limits the range of these species, the 20°C and 27°C mean annual temperature isotherms are also shown.



Pelaius platurus

57.14 A Block to Dispersal

The existence of the Isthmus of Panama prevents poisonous sea snakes from entering the Caribbean Sea from the Pacific Ocean.

1028 CHAPTER FIFTY-SEVEN

Biogeography and Human History

The distributions of land masses and species on Earth have had a strong influence on human history. The Old World happened to have a large number of species of plants and animals that were suitable for domestication. Eurasia was home to 39 species of large-seeded grasses, many more than were found in Africa or the Americas. Eurasia had 72 species of large mammals, compared with 51 in sub-Saharan Africa and 24 in the New World. Thirteen large mammal species, including pigs, horses, cattle, sheep, goats, and camels, were domesticated in Eurasia. None were domesticated in Africa, and only one, the llama, in the Americas.

To be amenable to domestication, large mammals needed to have three important social characteristics: They needed to live in herds, have well-developed male dominance hierarchies, and be nonterritorial. These traits enabled humans to tame the animals, exert dominance over them, and keep them in herds. All the large mammals of Africa lacked one or more of these traits. They have never been domesticated. The domesticated large mammals found in Africa today all came from Asia.

Domestication of large mammals had other important influences on human history. Many human diseases, such as smallpox and measles, were acquired from domesticated mammals. Eurasian people acquired immunity to these diseases. People on other continents did not. Thus when Europeans colonized the New World, they brought with them diseases that devastated the indigenous people—who transmitted no fatal diseases to the Europeans in turn. In addition, Europeans had horses, a domesticated mammal capable of carrying a person at high speeds. Horses have played a major role in human history, because cultures with horses have readily conquered cultures without them.

In the Old World, most mountain ranges are oriented in an east-west direction. Therefore, dispersal of people and their domesticated plants and animals was relatively easy, and dispersing individuals remained within climates with similar temperatures and day lengths. Humans dispersed into the New World only recently, across the high-latitude Bering land bridge. They brought with them no domesticated plants or animals. North America, as we have seen, had few species of grasses with large seeds. Maize, the grass that came to dominate American agriculture, was difficult to domesticate. Its eventual spread northward from its center of domestication in Mexico was possible only after extensive genetic changes that adapted the plants to the very different day lengths and climates of temperate North America.

Chapter Summary

► Biogeography is the science that attempts to explain patterns in the distribution of life on Earth.

Why Are Species Found Where They Are?

- ▶ If a species occupies an area, either it evolved there, or it evolved elsewhere and dispersed to that area.
- ▶ If a species is not found in a particular area, either it evolved elsewhere and never dispersed to that area, or it was once present in that area but no longer lives there.
- ▶ Continental drift has influenced the distributions of organisms throughout Earth's history.
- ▶ Ecological biogeographers seek to understand how current ecological interactions influence where species are found today.
- ▶ Historical biogeographers attempt to determine the influence of past events on today's patterns of species distributions.
- ▶ Historical biogeographers often analyze species distributions by converting phylogenies into "area phylogenies." Review Figure 57.1

The Role of History in Biogeography

- ▶ Biogeographers use the parsimony principle when they attempt to explain distribution patterns. Review Figure 57.2
- ▶ Vicariance and dispersal events have both influenced current distributions. Review Figure 57.3
- ▶ Animal biogeographers divide Earth into six major biogeographic regions. Plant biogeographers recognize two additional regions. Review Figure 57.4

Ecology and Biogeography

- ▶ Ecological biogeographers test theories that explain the numbers of species in different communities, how species disperse, and the effectiveness of barriers to movement.
- ▶ The island biogeographic model, which predicts the equilibrium species richness on islands, has been tested by examining patterns of distribution and by performing experiments. Review Figures 57.5, 57.6; Table 57.1
- ▶ The number of species in most lineages increases from polar to tropical regions. Review Figure 57.8

Terrestrial Biomes

- ▶ Terrestrial biomes are major ecosystem types that differ from one another in the structure of their dominant vegetation.
- ▶ The distribution of biomes on Earth is strongly influenced by annual patterns of temperature and rainfall. Review Figures 57.9, 57.10
- ▶ The major terrestrial biomes are tundra, boreal forest, temperate deciduous forest, temperate grassland, cold desert, hot desert, chaparral, thorn forest, savanna, tropical deciduous forest, and tropical evergreen forest.

Aquatic Biogeography

- ▶ No absolute barriers to the movement of marine organisms exist within the oceans, but most marine organisms have restricted ranges.
- ▶ Conditions in the oceans change dramatically with depth and sunlight penetration. Review Figure 57.11
- ▶ Boundaries between many pelagic regions are determined by ocean currents. Review Figure 57.12
- ▶ Species that live in shallow waters disperse with difficulty across wide deep-water barriers. Review Figure 57.13

Biogeography and Human History

- ▶ The distributions of plants, animals, and continents have exerted powerful influences on human history.

For Discussion

1. Horses evolved in North America, but subsequently became extinct there. They survived to modern times only

BIOGEOGRAPHY 1029

in Africa and Asia. In the absence of a fossil record, we would probably infer that horses originated in the Old World. Today, the Hawaiian Islands have by far the greatest number of species of fruit flies (*Drosophila*). Would you conclude that the genus *Drosophila* originally evolved in Hawaii and spread to other regions? Under what circumstances do you think it is safe to conclude that a group of organisms evolved close to where the greatest number of species live today?

2. The island biogeographic model we described incorporates almost nothing about the biology of the species involved. What traits of species should be incorporated into more realistic models of rates of colonization and extinction of species on islands?

3. Experiments to test theories of species richness are necessarily short-term. What long-term consequences of colonization and extinction are likely to be undetected by these experiments? How could they be studied?
4. In nearly every ecological community, the number of species present is much smaller than the number potentially available to colonize it. What inferences can be drawn from this pattern?
5. A well-known legend states that Saint Patrick drove the snakes out of Ireland. Give some alternative explanations, based on sound biogeographic principles, for the absence of indigenous snakes in that country.
6. Why are there so few species of trees in boreal forests? Why do few species of trees of boreal forests have animal-dispersed seeds?
7. What are some significant present-day human problems whose solutions involve biogeographic considerations? What kinds of biogeographic knowledge are most important for addressing each one?
8. Most of the world's flightless birds are either nocturnal and secretive (such as the kiwi of New Zealand) or large, swift, and well armed (such as the ostrich of Africa). The exceptions are found primarily on islands. Many flightless island species have become extinct with the arrival of humans and their domestic animals. What special biogeographic conditions on islands might permit the survival of flightless birds? Why has human colonization so often resulted in the extinction of such birds? The power of flight has been lost secondarily in representatives of many groups of birds and insects; what are some possible evolutionary advantages of flightlessness that might offset its obvious disadvantages?



Conservation Biology

When Polynesian people settled in Hawaii about 2,000 years ago, they exterminated—

probably by overhunting—at least 39 species

of land birds. Among them were 7 species of

Mallards, 2 species of flightless ibises, a sea eagle,

a small hawk, 7 flightless rails, 3 species of owls, 2 large

crows, a honeyeater, and at least 15 species of finches.

No people lived in New Zealand until about 1,000 years ago, when the Maori colonized the islands. Hunting by the Maori caused the extinction of 13 species of flightless moas, some of which were larger than ostriches.

When humans arrived in North America over the Bering land bridge, about 20,000 years ago, they encountered a rich fauna of large mammals. Most of those species were exterminated within a few thousand years. A similar extermination of large animals followed the human colonization of Australia, about 40,000 years ago. At that time Australia had 15 genera of marsupials larger than 50 kg, a genus of gigantic lizards, and a genus of heavy, flightless birds. All the species in 13 of those 15 genera had become extinct by 18,000 years ago.

The accelerating pace of human-caused extinctions of species, which raises serious concerns about the future of biological diversity on Earth, has led to the rapid development of the applied discipline of conservation biology—the scientific study of how to preserve the diversity of life. Conservation biologists study the causes of endangerment and extinction and develop methods to help preserve genes, species, communities, and ecosystems. The science of conservation biology draws heavily on concepts and knowledge from population genetics, evolution, ecology, biogeography, wildlife management, economics, and sociology. In turn, the needs of conservation are stimulating new research in those fields.

In this chapter we will see how biologists estimate rates of species extinction and the causes of endangerment, and learn how management plans can be used to reduce extinction rates and restore endangered species and communities to states in which they are likely to persist for a long time.

Extinct Flightless Hawaiian Birds

This artist's reconstruction of a flightless Hawaiian goose shows one of the many bird species exterminated by the Polynesian settlers of the islands.

Estimating Current Rates of Extinction

Most human activities that are causing extinctions are not new, but there are many more of us doing those things than ever before (see Figure 54.16). We have also added the results of our advancing technology, such as pesticides and climate change, to the array of pressures created by human activities.

We do not know how many species will become extinct during the next 100 years, first, because we do not know how many species there are on Earth, and second, because the number of extinctions will depend both on what we do and on

unexpected events. However, several methods exist for estimating probable rates of extinction resulting from human actions. In this section we will discuss how conservation biologists estimate current rates of extinction and identify species at risk of extinction.

Species-area relationships are used to estimate extinction rates

When we described the island biogeographic model in Chapter 57, we saw that the number of species on an island increases with the size of the island (see Figure 57.6). This species-area relationship can be applied to habitat patches



Green areas indicate dense forest cover.



58.1 Deforestation Rates Are High in Tropical Forests

Central America provides an example of the high rate of destruction of tropical forests that has taken place in recent years. As the forests are lost, so are the many species that live in them.

on mainlands as well. Conservation biologists often use the well-established relationship between the size of an area and the number of species present to estimate numbers of species extinctions resulting from habitat destruction.

The rate at which tropical forests are being logged and converted to cropland and pasture is not precisely known, but it is currently very high (Figure 58.1). These forests are Earth's richest biomes, home to perhaps one-half of all the species on the planet. Calculations using the species-area relationship applied to tropical forests are far from exact, but can result in estimates of over 1 million species extinctions in the next few decades.

Even the lowest estimates of current extinction rates predict that at least 10 percent of Earth's species are likely to become extinct during the next two decades. Some estimates predict extinction of 50 percent of Earth's species

CONSERVATION BIOLOGY 1031

during the next 50 years. Extinction rates have been much higher on islands than in mainland areas during the past 400 years (Figure 58.2), but extinction rates on continents are also rising fast.

Population models are used to estimate risks of extinction

To estimate the risk that a population will become extinct, conservation biologists analyze information about interactions between a population's genetic variation, morphology, physiology, and behavior and its environment, both physical and biological. Although rarity itself is not always a cause for concern, species whose populations are shrinking rapidly usually are at risk. Species with only a few individuals confined to a small range are likely to become extinct because they can be eliminated by local disturbances such as fires, unusual weather, disease, and predators.

Both demographic and genetic information were used in assessing the risk of extinction of Furbish's lousewort, a plant that is restricted to the banks of the St. John River in northern Maine. The Furbish's lousewort population is divided into discrete subpopulations growing at separate sites. These subpopulations are found where periodic disturbance, often caused by spring ice scour when blocks of floating ice scrape the stream banks, prevent establishment of trees and shrubs that would outcompete the louseworts (Figure 58.3). Data on annual rates of survival, growth, and reproduction were gathered by following more than 6,000 individually marked plants between 1983 and 1986. Extinctions of subpopulations and foundings of new subpopulations were estimated by counting plants over the entire range of the species.

Although Furbish's lousewort depends on regular disturbance to suppress the growth of shrubs and trees, distur-

Extinction rates are higher on islands than on the mainland.

Reptiles and amphibians ■ Mainland

Birds

Mammals

Mollusks

30 60 90 120 150

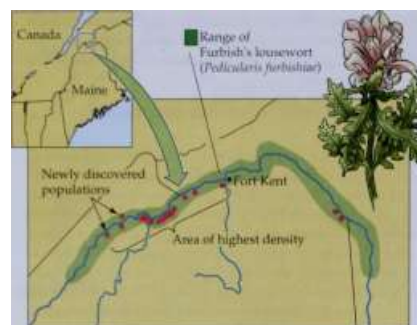
Number of extinctions, 1600-1990

180

58.2 Extinctions Have Been High on Islands

Between 1600 and 1990, extinctions of terrestrial vertebrates and mollusks were much higher on islands than on continents.

1032 CHAPTER FIFTY-EIGHT



58.3 Furbish's Lousewort Exists as a Metapopulation

Small populations of Furbish's lousewort exist along the St. John River, where ice scour and bank slumping eliminate shrubs and trees. Dispersing individuals colonize newly disturbed sites.

bances can also eliminate subpopulations. Between 1983 and 1984, three of ten study subpopulations were completely destroyed by ice scour and bank slumping, and none of the disturbed sites were recolonized during succeeding years. Thus if disturbance rates were too high, extinction rates of subpopulations are likely to be higher than rates of establishment of new subpopulations. Under current disturbance regimes, the two rates are about equal. Local subpopulations became extinct and new subpopulations were founded at an annual rate of about 3 percent during the study.

The investigators concluded that the persistence of Furbish's lousewort depends on disturbance events at sites currently having subpopulations and also at sites that are currently unoccupied. Clearly a strategy that protected only sites with current subpopulations would not maintain the species for many years. Preserving Furbish's lousewort depends on maintaining disturbance regimes along an entire stretch of the St. John River. If the St. John River continues to flow naturally and spring ice scouring continues, Furbish's lousewort is likely to persist for a long time.

Why Do We Care about Species Extinctions?

We care about species extinctions in part because humans depend on other species in many ways. For example, more than half the medical prescriptions written in the United States contain a natural plant or animal product (Figure 58.4). The search for and use of such products from the living world has hardly begun. Many species may be eliminated by tropical

forest destruction before we find out whether they might be sources of useful products.

Extinctions deprive us of opportunities to study and understand ecological relationships among organisms. The

more species are lost, the more difficult it will be to understand the rules that govern the structure and functioning of ecological communities.

We also derive enormous aesthetic pleasure from interacting with other organisms. Many people would consider a world with far fewer species as a less desirable one in which to live. Living in ways that cause the extinction of other species also raises serious moral and ethical issues that are receiving increased attention.

Ecosystem processes, as well as individual species, produce many benefits to humanity. Among them are the generation and maintenance of fertile soils, prevention of soil erosion, detoxification and recycling of waste products, regulation of hydrological cycles and the composition of the atmosphere, control of agricultural pests, pollination, and maintenance of the species richness upon which humanity depends. It is easy to list these ecosystem services, but to justify the allocation of scarce public resources to maintain them, we need quantitative estimates of their value.

A detailed study by economists, ecologists, and land managers in Western Cape Province, South Africa, has shown that an intensive program to eradicate invasive alien plants in the highlands of the region is a cost-effective way of maintaining a reliable regional supply of high-quality water. The native vegetation of these highlands is a species-rich community of shrubs, known as fynbos (pronounced "fainbos"). Fynbos can survive regular summer drought, nutrient-poor soils, and the fires that periodically sweep through the highlands (Figure 58.5a).

The fynbos-clad highlands provide about two-thirds of the Western Cape's water requirements. In addition, the flora is harvested for cut and dried flowers and thatching



Catharanthus roseus

58.4 Source of a Life-Saving Drug

A drug derived from the Madagascar rosy periwinkle has greatly increased the survival rate of children with leukemia. Other species that might be sources of drugs are being eliminated by deforestation on Madagascar.

(a)

CONSERVATION BIOLOGY 1033



(b) Stream flow from fynbos watersheds

c_g

3 T3

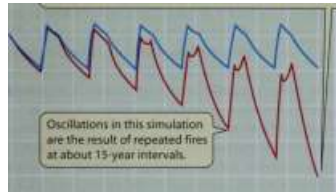
Relative biomass

(c) Computer simulation

380 r

340 -

As invasive species outcompete the native species and use more of the available water resources, runoff water will decrease by more than half in only 100 years.



Oscillations in this simulation are the result of repeated fires at about 15-year intervals.

j_

20

40 60

Years

80

100

grass. The combined value of these harvests in 1993 was about \$19 million. Some of the income from tourism in the region comes from people who want to see the fynbos vegetation. About 400,000 people visit the Cape of Good Hope Nature Reserve each year, primarily to see the many unique plants.

During recent decades, a number of plants introduced into South Africa have invaded the fynbos highlands. Be-

58.5 Water Flows from Fynbos

{a) The fynbos of South Africa, (b) Stream flow from fynbos watersheds is inversely proportional to plant biomass. (c) A computer simulation of stream flows from watersheds that have and have not been invaded by trees from outside the region.

cause they are taller and grow faster than the native plants, the exotics increase the intensity and severity of fires. By transpiring larger quantities of water, they decrease stream flows to less than half the amount flowing from mountains covered with native plants (Figure 58.5b,c).

Removing the exotic plants by felling and digging out invasive trees and shrubs and managing fire is estimated to cost between \$140 and \$830 per hectare, depending on the densities of invasive plants. Annual follow-up operations will cost about \$8 per hectare. The costs of alternative methods to replace the water lost from watersheds taken over by exotic plants are much higher. A sewage purification plant that would deliver the same volume of water as a well-managed watershed of 10,000 hectares would cost \$135 million to build and \$2.6 million per year to operate. Desalination of seawater would cost four times as much. Thus, the available alternatives would deliver water at a cost between 1.8 and 6.7 times more than the costs of maintaining natural vegetation in the watershed.

Modern industrial societies often favor technologically sophisticated methods of substituting for lost ecosystem services. The study of water resources in the Western Cape Region shows that simple but labor-intensive methods—cutting and burning—can be cheaper. In addition, they preserve other ecosystem values, such as tourism and commercial plant products.

Some ecosystem values, such as aesthetic benefits, cannot be replaced with technological inventions. Aesthetic benefits may contribute much to a country's economy. One of the largest sources of foreign income in Kenya is nature tourism. The loss of a single species probably would not reduce the flow of tourists to Kenya, but if elephants, rhinoceroses, lions, leopards, and buffalo were all to disappear, fewer people would pay the high price of a Kenyan vacation. Populations of these species can be maintained only if large tracts of the ecosystems in which they live are preserved.

Determining Causes of Endangerment and Extinction

Rare species are more likely to become extinct than common species. Species may be rare for any of several reasons. They may live in a habitat that is rare, such as desert lakes with high salt concentrations or caves (Figure 58.6). Another reason for rarity is trophic level—secondary carnivores are usually rare because so little energy is available to support their populations,

as we saw in Chapter 56. Being rare increases a species' chance of becoming extinct, but common species can also become extinct.

1034 CHAPTER FIFTY-EIGHT

Red dots show subterranean "hot spots" of biodiversity.



The dark areas indicate distribution of karst. Suitable caves are sparsely distributed within these regions.

Freshwater mussels

Crayfishes

Amphibians

Freshwater fishes

Flowering plants

Conifers

Ferns

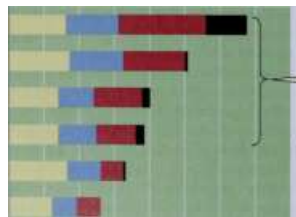
Tiger beetles

Dragonflies, damselflies

Reptiles

Butterflies, skippers

Mammals Birds



Small animals are vulnerable to habitat destruction because they often have small ranges and - | highly specialized requirements.

58.6 The Habitat of Cave Animals Is Patchy and Restricted

The map shows the distribution of subterranean limestone (karst) in which caves with running water are found. The caves that can actually be inhabited by cave animals covers a much smaller area.

An analysis by The Nature Conservancy revealed that habitat loss is the most important cause of endangerment of species in the United States (Figure 58.7), but that other factors, such as exotic species, pollution, overexploitation, and disease, also threaten species. In this section we will examine some of these major threats to species.

Habitat destruction and fragmentation are important causes of extinction today

The 6 billion humans that live on Earth today are fed, clothed, and housed by the agricultural and forestry industries, which convert natural ecological communities containing many species into highly modified communities dominated by one or a few species of plants. Within these communities, humans discourage the presence of other species by killing competing plants, bacteria, fungi, nematodes, insects and other arthropods, and vertebrates.

Although agricultural ecosystems have always harbored fewer species than the complex natural ecosystems they replaced, only recently have farmers planted large tracts of land in single crops. Traditional farmers planted many different crops together, maintaining some of the diversity that is key to the functioning of natural communities. Many species that cannot survive in intensive modern agricultural systems live in traditional agricultural ones. In traditional coffee plantations, for example, coffee bushes are grown in the shade of large trees (Figure 58.8f). These structurally rich plantations support populations of many species of birds, and few pesticides need to be applied to them. Some recently developed high-yielding strains of coffee, however, grow best in full sunlight. Pure plantations of these coffee bushes require heavy applications of pesticides and support almost no birds (Figure 58.8b).

Agriculture and forestry today are so extensive that more than 30 percent of all net terrestrial primary production is diverted for human use. All other species on Earth must survive on only two-thirds of the total global terrestrial production, and the fraction people divert is steadily increasing.

As natural habitats are progressively destroyed, the remaining patches increasingly become smaller and more isolated. Small habitat patches are qualitatively different from larger patches of the same habitat in ways that affect the

Habitat destruction is the primary cause of extinction and endangerment for freshwater aquatic organisms.

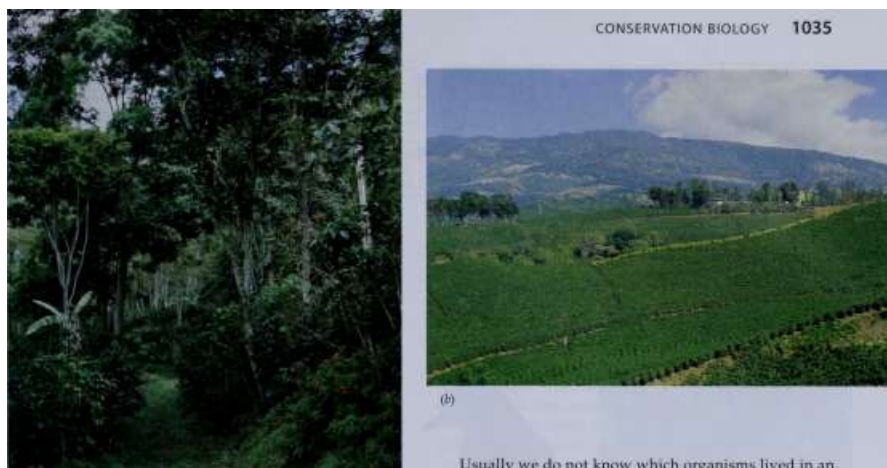
10 20 30 40 50 Percent of species

60 70

80

58.7 Proportion of U.S. Species Extinct or at Risk

The groups that are most endangered—mussels, crayfishes, amphibians, and fishes—live in fresh waters, a habitat that has been extensively destroyed and polluted.



(«)

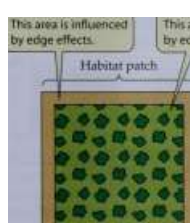
58.8 The Way Coffee is Grown Affects Biodiversity

(a) A traditional coffee plantation with a canopy of trees supports many species of birds, (b) Few bird species live in plantations of sun-grown coffee.

survival of species. Small patches cannot maintain populations of species that require large areas, and they support only small populations of many of the species that can survive in them.

In addition, the fraction of a patch that is influenced by conditions in adjacent habitats—resulting in edge effects — increases rapidly as patch size decreases (Figure 58.9). Close to the edges of forest patches, for example, winds are stronger, temperatures are higher, humidity is lower, and light levels are higher than they are farther inside the forest. Species from surrounding habitats often colonize the edges of patches to compete with or prey upon the species living there.

This area is influenced by edge effects.



This area is not influenced by edge effects.

Because the width of the edges is relatively constant, as the total area becomes smaller, the edge becomes proportionately

larger.



Usually we do not know which organisms lived in an area before its habitats became fragmented. To address this problem, a major research project near Manaus, Brazil, was launched in an area of tropical forest before logging took place. The landowners agreed to preserve forest patches of certain sizes and locations (Figure 58.10V?).

Biologists conducted censuses of those patches while the areas were still part of the continuous forest. Soon after the surrounding forest was cut, species began to disappear from the isolated patches. The first species to be eliminated were monkeys with large home ranges, such as the black spider monkey, the tufted capuchin, and the bearded saki, and antbirds that follow raiding army ant swarms to capture insects flushed by the ants (Figure 58.10b,c).

Species that become extinct in small fragments are unlikely to become reestablished because individuals dispersing from other locations are less likely to find isolated patches. Even if they find them, the patches may be too small to support their populations on a long-term basis. The persistence of species in small patches may be improved if the patches are connected by corridors of suitable habitat through which individuals can disperse.

The role of corridors in sustaining populations of species was studied by creating patches of mosses on the surface of a large rock. Eight experimental "landscapes" were established by scraping away the moss to create patches of equal size. Some patches were isolated, others were connected by narrow moss corridors, and still others were connected by pseudocorridors

□

30.55%

43.75%

64%

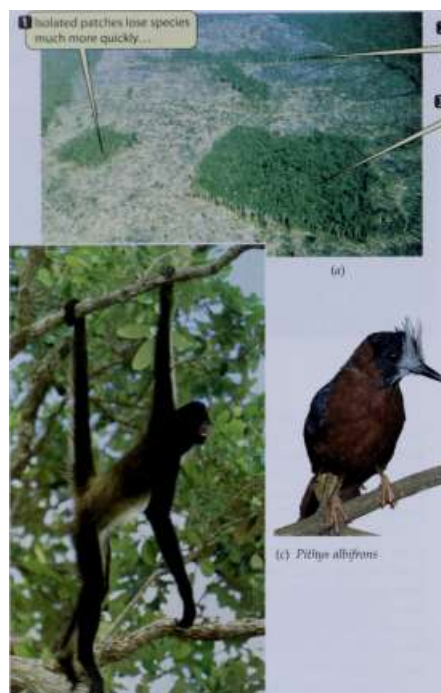
.8% - ►

Increasing percentage of patch influenced by edge effects

58.9 Edge Effects

The smaller a habitat patch, the greater the proportion that is influenced by conditions in the surrounding environment.

1036 CHAPTER FIFTY-EIGHT



(b) Ateles sp.

58.10 Brazilian Forest Fragments and Species Loss

(a) This tropical forest near Manaus, Brazil, was studied before and after logging to determine the effects of habitat fragmentation on species diversity, (b) Spider monkeys, which require a large range, quickly disappeared from isolated forest

patches, (c) The white-plumed antbird, a species common in Brazilian forests, has become extinct in isolated forest plots, but survives in patches connected to continuous forest habitat.

that were interrupted by a 10 mm break (Figure 58.11). The abundance and species richness of tiny arthropods were measured at 2-month intervals over the course of a year—a time period equivalent to many generations for most of the small animals living in the moss.

Remarkably/ barriers as narrow as 10 mm were sufficient to greatly reduce the dispersal rates of arthropods between moss patches. Isolated patches and patches connected by pseudocorridors had only about 60 percent as many species of springtails (tiny, wingless insects) and mites as the patches connected by complete corridors.

...than patches connected to the main forest.

Even larger patches are strongly influenced by edge effects.

EXPERIMENT

Question: What effect does habitat fragmentation have on number of species?

METHOD Ecosystem studied: small organisms (mostly

arthropods) living in patches of moss on rocks. Moss areas on rocks are trimmed to form distinct patches.

Control Experiment 1 Experiment 2 patches patch

Moss patches



Experiment 1

50 cm



•*— 50 cm—*► Control patch

Fragments, each 20 cm²

RESULTS

In fragments, 40% of species went extinct after 1 year.

Experiment 2

10-mm gaps

50 cm



■<—50 cm—► Control patch

RESULTS

Fragments connected by 7-cm corridors

14% of the species went extinct after

6 months.

Fragments

connected by

pseudocorridors

with gaps

41% of the species went extinct after

6 months.

Conclusion: Even small barriers to dispersal can raise extinction rates.

58.11 An Experiment Demonstrates the Value of Corridors

A small-scale experiment in which patches were created by removing moss from a rock surface demonstrated that even small gaps between suitable habitats can reduce the number of species that persist in patches.



58.12 Galapagos Tortoises Are Reared in Captivity

Conservationists at the Charles Darwin Research Station remove tortoise eggs from nests. When the eggs hatch, the young are reared in captivity until they are large enough to be invulnerable to predation by introduced pigs and rats. Populations of tortoises on some Galapagos islands would be extinct if they were not propagated in captivity.

CONSERVATION BIOLOGY 1037

cause by removing most of the algae from the water, thereby clarifying it, zebra mussels allow sunlight to penetrate more deeply into the water column. As a result, in areas of high mussel densities, populations of submerged vascular plants and some invertebrates have increased. Not until after many years will we know the full effects of the colonization of North America by zebra mussels.

Some pests proliferated quickly following their introduction to new continents, with destructive consequences. Forest trees in eastern North America, for example, have been attacked by several European diseases. The chestnut blight, caused by a European fungus, virtually eliminated the American chestnut, formerly a dominant tree in forests of the Appalachian Mountains. Nearly all American elms over large areas of the East and Midwest have been killed by Dutch elm disease (caused by a different fungus), which reached North America in 1930. Ecologists suspect that intercontinental movement of disease organisms has caused extinctions throughout life's history, but evidence of disease outbreaks is not usually preserved in the fossil record.

Introduced pests, predators, and competitors have eliminated many species

Deliberately and accidentally, people move many organisms from one continent to another. Pheasants and partridges were introduced into North America for hunting. Europeans introduced rabbits and foxes to Australia for sport. Many plants have been introduced as ornamentals. Weed seeds have been accidentally carried around the world in soil used as ballast in sailing ships and as contaminants in sacks of crop seeds. Despite quarantines, disease organisms have spread widely, carried by infected plants, animals, and people.

A species that has evolved over time in a community with certain predators and competitors may be driven to extinction by newly introduced predators and competitors. Nearly half of the small to medium-sized marsupials and rodents of Australia have been exterminated during the last 100 years by a combination of competition with introduced rabbits and predation by introduced cats and foxes. On the Galapagos archipelago, introduced pigs and rats had exterminated several races of tortoises even before Darwin visited the islands. Populations of tortoises on some islands are maintained today only because conservationists remove eggs and rear the young tortoises in captivity (Figure 58.12).

The zebra mussel, whose larvae were carried in ships' ballast water from Europe, became established in the Great Lakes in about 1985. Zebra mussels dispersed rapidly and today occupy much of the Great Lakes and Mississippi River drainage (Figure 58.13). In some places these mussels have reached densities as high as 400,000 per square meter! Some species of native clams are being covered and smothered by zebra mussels. Zebra mussels have also caused millions of dollars of damage to water intake structures. On the other hand, some native species have benefited, be-

I Zebra mussels entered North American waters when ballast water from European ships was pumped into Lake Ontario.



I The mussels became established and rapidly spread via rivers through eastern North America.

58.13 Introduced Zebra Mussels Spread Rapidly

Between 1986 and 1988 the range of zebra mussels in North America nearly doubled. This introduced species spreads rapidly because zebra mussel larvae are free-swimming and adults can attach to moving objects, such as boat hulls.



Dreissena polymorpha

1038 CHAPTER FIFTY-EIGHT

The curved bill of the iiwi matches the shape of the Lobelia flower.



58.74 Coevolved Mutualists

Declining populations of the iiwi (*Vestiaria coccinea*), a Hawaiian honeycreeper, also threaten the Lobelia plant, which has no other pollinator.

Overexploitation has driven many species to extinction

Until recently, humans caused extinctions primarily by overhunting. The passenger pigeon, the most abundant bird in North America in the early 1800s, became extinct by 1914, largely due to overhunting. Russian whalers exterminated the unusual Steller's sea cow of the North Pacific in the late 1800s, just 37 years after it was first described. Such overex-

(a)



If the climate of eastern North America warms by as little as 4°C, about half the potential future range of beech trees will be beyond the northernmost extent of the current range.



Exploitation continues today. Elephants and rhinoceroses are threatened in Africa because poachers kill them for their tusks and horns. Unfortunately, these animals are not slaughtered for medical or other useful purposes; rather, they are killed for ornaments and because some men believe that powdered horn enhances their sexual potency. Many species of orchids, parrots, reptiles, and tropical reef fishes are currently threatened by lucrative pet and houseplant trades.

Loss of mutualists threatens some species

Many plants have mutualistic relationships with pollinators, but most of these mutualisms are not highly species-specific. On islands, however, where ecological communities contain relatively few species, plant-pollinator interactions often evolve to be highly specific. For example, a single species of the plant *Lobelia* colonized the Hawaiian Islands, where it eventually gave rise to 110 daughter species. A single colonizing species of songbird gave rise to at least 47 species of Hawaiian honeycreepers, some of which have long, slender, curved bills. These nectar-feeding birds were the only pollinators of many species of Hawaiian lobelias (Figure 58.14).

Today, half of the nectar-feeding birds of Hawaii are extinct, leaving many lobelias without pollinators. Many of these lobelias still survive, but populations of some species have been reduced to only a few individuals. A few species survive only because biologists artificially pollinate them.

Global warming may cause species extinctions

Atmospheric scientists predict that, as a result of increasing concentrations of CO₂ and other greenhouse gases in the atmosphere (see Chapter 56), average temperatures in North America will increase 2°-5°C by the end of the twenty-first century. If the climate warms by only 1°C, the average temperature currently found at a certain location will shift 150 km to the north. To remain in the temperature regime to which they are accustomed, species will have to shift their ranges 150 km to the north. Species will need to shift their ranges as much as 500-800 km in a single century if the climate warms 2-5°C. Some habitats, such as alpine tundra, could be eliminated from many areas as forests expand up the mountain slopes.

58.15 Threatened by Global Warming

(a) Seedlings and saplings abound in this beech forest, (b) Beeches would need to migrate 40 times faster than they have in the past to keep up with the anticipated rates of climate change.

Conservation biologists are attempting to predict the effects of global warming on North American species. Trees might be especially vulnerable to climate change because they grow for long periods before they begin to reproduce, and their seeds typically move only very short distances (Figure 58.15).

If Earth warms as predicted, climatic zones will not simply shift northward. New climates will develop, and some existing climates will disappear. New climates are certain to develop at low elevations in the tropics because a warming of even 2°C would result in climates near sea level that are hotter than those found anywhere in the humid tropics today. Adaptation to those climates may prove difficult for many tropical organisms.

Preventing Species Extinctions

Designing recovery plans

Once the causes of endangerment of species have been identified, appropriate remedies can be designed. In the United States, when a species is listed as threatened or endangered under the Endangered Species Act, a recovery plan is typically prepared to guide efforts to improve the status of the species. In this section we will describe how good diagnoses have been used to design management actions to prevent species from becoming extinct.

kirtland's warbler. The Kirtland's warbler is an endangered bird that nests only in 8-18-year-old stands of jack

Areas with sandy soils are absent north of the current breeding range.

Breeding range of Kirtland's warbler



(a) *Dendroica kirtlandia*

(b)

58.16 Kirtland's Warbler Is Threatened by Habitat Loss

(a) A male Kirtland's warbler in a young jack pine, (b) The warbler's breeding range and the distribution of the sandy soils that support stands of jack pine.

pine growing on sandy soils in Michigan (Figure 58.16). The current population of Kirtland's warblers is less than 1,000 individuals. Field studies determined that the Kirtland's warbler is at risk from both loss of habitat and nest parasitism by brown-headed cowbirds. Fire suppression has reduced the area of young jack pine stands, and cowbirds, which lay their eggs in other birds' nests, have greatly increased in abundance in the area. To prevent further threats to the warblers, conservation biologists ignite controlled fires in jack pine forests to maintain a steady supply of trees of the right age. They are also removing brown-headed cowbirds to reduce nest parasitism rates.

the California sea otter. Populations of the California sea otter were hunted nearly to extinction during the nineteenth century. After receiving legal protection in 1911, the species increased steadily to about 2,400 individuals today. The Southern Sea Otter Recovery Team was charged with developing a recovery plan for the sea otter under the U.S. Endangered Species Act. They determined that ample habitat and food supplies are present and that the otters are reproducing and surviving well. They judged that a major oil spill poses the most serious threat to the otters.

A demographic model suggested that the otter population would be endangered if it dropped below 1,850 individuals. A model designed to assess whether a major oil spill could reduce the population to that size suggested that fewer than 800 otters would be killed by 90 percent of the simulated spills. Therefore, the team set the "delisting" criterion at 2,650 individuals (1,850 + 800). Because the California sea otter population has nearly reached this size, it may soon be removed from the list of endangered species.

Captive propagation has a role in conservation

Species being threatened by overex-ploitation, loss of habitat, or environmental degradation through pollution can sometimes be maintained in captivity while the external threats to their existence are reduced or removed. Captive propagation is only a temporary measure that buys time. Existing zoos, aquariums, and botanical gardens do not have enough space to maintain adequate populations of more than a small fraction of Earth's rare and endangered species. Nonetheless, captive propagation can play an important role by maintaining species during critical periods and by providing a source of individuals for reintroduction into the wild. Captive propagation projects in zoos also have raised public awareness of species that are threatened with extinction.

1040 CHAPTER FIFTY-EIGHT

58.77 Peregrine Falcon Populations Have Been Reestablished

(a) Peregrine falcons have responded well to captive propagation. Some individuals have adapted to urban life, nesting on the tall buildings of cities and feeding on pigeons, (b) Throughout the eastern United States, many pairs of peregrine falcons now

attempt to reproduce. Most of them are successful.

100 -

80 -

01

i-

at

a.

•8 60

■ S 40

s

20

1980

(b)

THE PEREGRINE FALCON. In 1942,

about 350 pairs of peregrine falcons bred in the United States east of the Mississippi River. This breeding population disappeared entirely by 1960. The cause of the falcon's disappearance was the widespread use of organochlorine pesticides, such as DDT and dieldrin. These pesticides degrade very slowly in the environment and become concentrated in the falcon's prey. Their accumulation in the peregrines' bodies interfered with the deposition of calcium in eggshells. As a result, most of the falcons' eggs broke before they hatched.

Much of the eastern United States became suitable habitat for peregrines again after the use of DDT in the United States was banned by federal law. Captive breeding of peregrines began at Cornell University in 1970, and by the end of 1986, more than 850 birds reared in captivity had been released in 13 eastern states, with spectacular success (Figure 58.17). Peregrines probably would have recolonized the East by themselves, but they would have done so much more slowly without human assistance.

the California condor. With its 9-foot wing span, the California condor is North America's largest bird. Two hundred years ago, condors ranged from southern British Columbia to northern Mexico, but by 1978, the wild population was plunging toward extinction—only 25 to 30 birds remained in southern California. To save the condor from extinction, biologists initiated a captive propagation program in 1983.

The first chick conceived in captivity hatched in 1988. By 1993, nine captive pairs were producing chicks, and the captive population had increased to more than 60 birds. The captive population was large enough that six captive-bred birds could be released in the mountains north of Los Angeles in 1992. These birds are provided with contaminant-free food in remote areas, and they are using the same roosting sites, bathing pools, and mountain ridges as did their predecessors. Captive-reared birds also were released late in 1996 in northern Arizona. It is still too early to pronounce the program a success, but without captive propagation, the California condor would probably be extinct today

The cost of captive propagation is comparatively low

The California condor rehabilitation program costs about 1 million dollars a year. The Peregrine Fund at Cornell Uni-



1982

1984 Year

1986 1988

1990

Pairs producing

offspring versify spent about 3 mil-

lion dollars over the past 30 years; the expenses of other cooperating agencies add at least another half million to the total. These amounts may seem large, but they are small compared with the costs of other human activities; for example, even a minor Hollywood film costs more than this to produce, and such films often lose money.

Establishing Priorities for Conservation Efforts

Many species and ecosystems are threatened, but the financial and human resources that can be allocated to preservation efforts are limited. How should those resources be spent to achieve the most conservation benefits? Because many species can survive only in the ecological communities in which they evolved, preserving the full array of ecological communities and habitats is vital.

Where should parks be established?

Parks, sanctuaries, and reserves function to maintain species and ecosystems relatively free of human disturbance. Parks are being created in many countries, but where should they be established to achieve the greatest conservation benefits?

High value sites for parks and reserves are those that

- Are home to unusually large numbers of different species.
- Have many endemic species—species that originated in that region and usually are found nowhere else.

Areas of high endemism should receive high conservation priority because if the endemic species are lost there, they often become globally extinct. Madagascar is a good example of such center of endemism: Nearly all the vascular plants and vertebrates of Madagascar are found only on that island (Figure 58.18). Therefore, if the small fragments of tropical and subtropical forests remaining on Madagascar were destroyed, many species would be exterminated.

CONSERVATION BIOLOGY

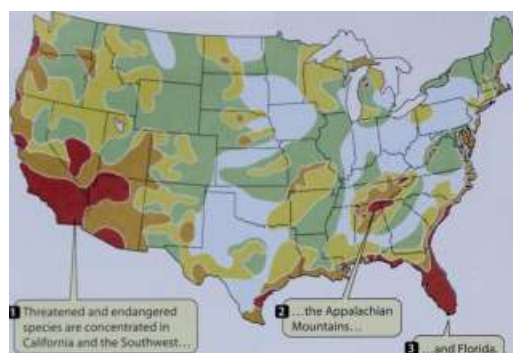


Eulemur fulvus (brown lemur)

58.18 Madagascar Abounds with Endemic Species

The majority of plant and animal species found on the island of Madagascar, off the eastern coast of Africa, are found nowhere else on Earth.

1042 CHAPTER FIFTY-EIGHT



| Threatened and endangered species are concentrated in California and the Southwest...

None <40 40-80 80-95 >90

Centers of endemism are not the same for all groups of organisms. Nevertheless, some areas have high concentrations of endangered species in many taxa. In the United States, species listed as threatened and endangered under the Endangered Species Act are concentrated in California, the Southwest, Florida, Hawaii, and the Appalachian Mountains (Figure 58.19).

Some economic land uses are compatible with conservation

In most countries, new parks and reserves must be established in already settled areas because few pristine areas remain. The people living there cannot be evicted, nor is it appropriate, in most cases, to prevent hungry people from settling in or hunting in parks. The high rates of human population growth in most tropical countries guarantee that pressures on parks from agricultural settlers will increase rather than decrease.

For these reasons, lands that are exploited for food, medicines, and fiber must play an important role in conservation. These lands are far more extensive than parks and reserves, and they include ecosystems not represented in parks. Fortunately, many species can be preserved on lands that are being used for economic purposes. Only a few species, such as predators on humans and domestic animals or large, destructive herbivores, are incompatible with most human uses of land.

Forest reserves in which economically valuable products are harvested can support both species preservation and economic development. In Belize, people known as hier-bateros collect medicinal plants in the forests and sell them to the curanderos (healers) who provide 75 percent of health care in the country. A botanist and an economist determined that two 1-hectare plots in the second-growth tropi-

58.19 Geographic Distribution of Threatened and Endangered Species in the United States

Threatened and endangered species are concentrated in just a few locations in the continental United States. These areas are obvious priorities for conservation efforts.

cal forest of Belize yielded gross annual revenues of \$865 and \$4,017, which are greater than the incomes that would result from cultivating squash and corn on those plots.

Conservation requires large-scale planning

Scientists at the World Wildlife Fund have developed a large-scale approach called Ecoregion-Based Conservation (ERBC). An ecoregion is an area that has a relatively uniform climate and a biota dominated by a group of widely distributed species. Using all available information on species' distributions and ecological requirements, scientists can identify sites of highest conservation priority within the ecoregion. This information is then used to develop a vision of what successful conservation would look like in 50 years.

An ecoregional conservation project, known as the Yellowstone to Yukon Initiative (Y2Y), is under way in Canada and the northern United States. Y2Y is a binational effort to restore and maintain biological diversity and landscape continuity in an area encompassing 1.2 million square kilometers along the spine of the Rocky Mountains. Information on the distributions of species, human cultures, and soil types in the region is being used to identify the most important areas for maintaining biological diversity. Investigators have also identified threats to biodiversity resulting from mining and its associated toxic waste pollution, unsustainable logging, and conversion of large ranches to suburban developments and sites for summer homes.

An important product of the Y2Y Initiative has been identification of areas that must be preserved or restored as key corridors for the movement of large mammals, such as grizzly bears, wolves, and elk, within the ecosystem. Efforts are now under way to build the base of political and financial support that will be necessary to make the vision a reality.

Restoring Degraded Ecosystems

Many areas that could be incorporated into reserves have been highly altered by human activities. Some of these areas can play their intended roles in biodiversity conservation only if they are restored to their original state. To accomplish this task, a subdiscipline of conservation biology, known as restoration ecology, is growing rapidly. Research on methods of restoring populations, communities, and

CONSERVATION BIOLOGY 1043

ecosystems is needed because many ecological communities will not recover, or will do so only very slowly, without creative intervention in the recovery process.

The world's largest restoration project is under way in Guanacaste National Park in northwestern Costa Rica. Its goal is to restore a large area of tropical deciduous forest—the most threatened ecosystem in Central America—from small fragments that remain in an area converted mostly to pastures.

The single most important threat to Guanacaste National Park is fires, most of which are started by people. These fires burn the introduced pasture grasses and spread far into surrounding forests. Grazing by domestic livestock lowers the densities of these grasses, and the animals also disperse the seeds of native trees that can invade pastures. Therefore, the restoration program encourages some initial grazing by domestic livestock in the park. When plant succession has progressed to the point where grass no longer poses serious competition to the woody species and is no longer sufficiently dense to carry hot fires, grazing is terminated.

Restoring damaged and degraded habitats is an important activity, but ecologists still have limited ability to restore natural ecosystems. In the United States, the self-serving but false belief that comparable ecosystems can be created somewhere else has made it easy to get building permits for developments that destroy habitats. Developers need only state that they will create substitutes for the ecosystems they are destroying, but promising to do this is much easier than doing so.

Even the most experienced wetland ecologists are having great difficulty creating new wetlands that mimic those being

destroyed. Such a "restored" wetland was conceived as part of a compensation agreement that allowed the California Department of Transportation to widen Interstate Highway 5 near San Diego. Despite stringent, court-imposed standards and the involvement of wetland experts, local endangered birds were still not breeding in the "restored" marsh 12 years after it was created. Therefore, as noted by a recent National Research Council committee on wetland restoration: "Wetland restoration should not be used to mitigate avoidable destruction of other wetlands until it can be scientifically demonstrated that the replacement ecosystems are of equal or better functioning."

Markets and Conservation

Most species are common property resources that are "owned" by everyone. Because no individual or group of individuals has strong incentives to use common property resources in a sustainable manner, their preservation usually depends on central governments. Unfortunately, governments generally lack sufficient resources to do the job. Also, governmental actions often are not well attuned to local situations. For these reasons, allowing local people to receive the economic benefits from managing biological resources on their lands can, under proper conditions, assist conservation efforts.

Preserving genes, species, and habitats provides many economic benefits. However, many of these benefits will assist future generations, not the current one. We do not know how future generations will value these benefits or the biodiversity that generates them. We do not even know how the current generation values them. We also cannot predict which species will turn out to be sources of valuable foods, medicines, or drugs.

Between 1951 and 1981, the National Cancer Institute screened extracts from 35,000 different species. To date, only one compound—taxol, derived from the Pacific yew tree—has received regulatory approval as a drug component. That drug is very valuable, but many species had to be searched to find it.

Although it is unlikely that any given species will have market value, extinction is forever. If we purposely or inadvertently exterminate a species, we have irreversibly destroyed a resource of unknown value. Therefore, loss of biodiversity is an especially urgent public issue.

How much should societies invest to preserve biodiversity? This question does not have a scientific answer. Economists and evolutionary biologists can contribute valuable information to the public debate, but the final decision is an ethical and political one that will depend on our beliefs about our responsibilities to the other organisms that share Earth with us.

The preservation of biological diversity and ecosystem services is one of the greatest challenges facing humankind. Many of the scientific tools needed for the task are already available, but appropriate use of these tools requires major changes in people's attitudes toward other species. If species are valued only because they are economically useful, increased losses of species are inevitable. Only when we value biological diversity and ecosystem functioning as the heritage of all humankind, a heritage to be passed on to our descendants as completely as possible, will we begin to reduce the current alarming rates of ecosystem destruction and species extinction.

©Chapter Summary Estimating Current Rates of Extinction

- ▶ Estimates of current rates of extinction worldwide are based primarily on species-area relationships and rates of tropical deforestation. Review Figure 58.1
- ▶ Rates of extinction are much higher on islands than on the mainland. Review Figure 58.2
- ▶ Demographic and genetic information is used to estimate risks of extinction. Review Figure 58.3

Why Do We Care about Species Extinctions?

- ▶ Diverse species provide the food, fiber, medicines, and aesthetic benefits upon which human life depends.
- ▶ Ecosystems provide services that can be replaced only by expensive and continuing human effort. Review Figure 58.5

Determining Causes of Endangerment and Extinction

- ▶ Rare species are the most vulnerable to extinction, but common species can also become extinct.

1044 CHAPTER FIFTY-EIGHT

- ▶ Habitat destruction is the most important cause of species extinction today, but overexploitation, which historically resulted in most human-caused extinctions, is still an important cause of extinctions. Review Figure 58.7

- ▶ [The fragmentation of habitats into patches that are too small to support populations is a major cause of extinction.

- ▶ [The proportion of a habitat patch subject to detrimental

effects increases as patch size decreases. Review Figure 58.9

- ▶ Exotic predators, competitors, and diseases introduced by humans are major causes of extinction. Review Figure 58.13

- ▶ In the future, global warming may be an important cause of extinction. Review Figure 58.15

Preventing Species Extinctions

► To ensure the recovery of endangered species, human manipulation of their environments and the other species that inhabit them is sometimes needed. Review Figure 58.16

► Captive propagation plays a useful role in conservation. Review Figure 58.17

Establishing Priorities for Conservation Efforts

► The best way to maintain populations is to set aside areas in which species and their habitats are protected. High-priority areas for establishing parks and reserves are regions of unusually high species richness and endemism. Review Figure 58.19

► In most countries, new parks must be created in already settled areas. Management of lands that are exploited for food, medicines, and fiber, must play an important role in conservation.

Restoring Degraded Ecosystems

► Restoration is an important component of recovery plans, but restoration of some types of environments, especially aquatic ones, is difficult.

Markets and Conservation

► Properly employed, markets can help preserve biodiversity.

► The conservation of biodiversity is not just a scientific or economic issue. It raises serious moral and ethical concerns that define what it means to be a human being on Earth.

For Discussion

1. Most species driven to extinction by people in the past were large vertebrates. Do you expect this pattern to persist into the future? If so, why? If not, why not?
2. Species endangered as a result of global warming might be preserved if we could move individuals from areas that are becoming unsuitable for them to those likely to be better for them in the future. What are the major difficulties associated with such interventions? For what types of species might they work well? Poorly? Make no difference?
3. Conservation biologists have debated extensively which is better: many small reserves or a few large ones. What ecological processes should be evaluated in making judgments about size and location of reserves? To what extent should we be concerned with preserving the largest number of species rather than those species judged to be of unusual importance for scientific, aesthetic, or commercial reasons?
4. During World War I, French doctors adopted a "triage" system of dealing with wounded soldiers. The wounded were divided into three categories: those almost certain to die no matter what was done to help them, those likely to recover even if not assisted, and those whose probability of survival was greatly increased if they were given medical attention. The limited resources available to the doctors were directed primarily at the third category. What would be the implications of adopting a similar attitude toward species preservation?
5. Economic arguments dominate discussions about the importance of preserving the biological richness of the planet. In your opinion, what role should moral arguments play?

Appendix:

Some Measurements Used in Biology

Glossary

Abdomen (ab' dun mun) [L.: belly] • In arthropods, the posterior portion of the body; in mammals, the part of the body containing the intestines and most other internal organs, posterior to the thorax.

Abscisic acid (ab sighs' ik) [L. abscissio: breaking off] • A plant growth substance having growth-inhibiting action. Causes stomata to close.

Abscission (ab sizh' un) [L. abscissio: breaking off] • The process by which leaves, petals, and fruits separate from a plant.

Absolute temperature scale • Also known as the Kelvin scale. A temperature scale in which zero is the state of no molecular motion. This "absolute zero" is -273° on the Celsius scale.

Absorption • (1) Of light: complete retention, without reflection or transmission. (2) Of liquids: soaking up (taking in through pores or cracks).

Absorption spectrum • A graph of light absorption versus wavelength of light; shows how much light is absorbed at each wavelength.

Abyssal zone (uh biss' ul) [Gr. abyssos: bottomless] • That portion of the deep ocean floor where no light penetrates.

Accessory pigments • Pigments that absorb light and transfer energy to chlorophylls for photosynthesis.

Acetylcholine • A neurotransmitter substance that carries information across vertebrate neuromuscular junctions and some other synapses. Acetylcholinesterase is an enzyme that breaks down acetylcholine.

Acetyl CoA (acetyl coenzyme A) •

Compound that reacts with oxaloacetate to produce citrate at the beginning of the citric acid cycle; a key metabolic intermediate in the formation of many compounds.

Acid [L. acidus: sharp, sour] • A substance that can release a proton in solution. (Contrast with base.)

Acid precipitation • Precipitation that has a lower pH than normal as a result of acid-forming precursors introduced into the atmosphere by human activities.

Acidic • Having a pH of less than 7.0 (a hydrogen ion concentration greater than 10^{-7} molar).

Acoelomate • Lacking a coelom.

Acquired Immune Deficiency Syndrome •

See AIDS.

Acrosome (a' krow soam) [Gr. akros: highest or outermost + soma: body] • The structure at the forward tip of an animal sperm which is the first to fuse with the egg membrane and enter the egg cell.

ACTH (adrenocorticotropin) • A pituitary hormone that stimulates the adrenal cortex.

Actin [Gr. aktis: a ray] • One of the two major proteins of muscle; it makes up the thin filaments. Forms the microfilaments found in most eukaryotic cells.

Action potential • An impulse in a neuron taking the form of a wave of depolarization or hyperpolarization imposed on a polarized cell surface.

Activating enzymes (also called aminoacyl-tRNA synthetases) • These enzymes catalyze the addition of amino acids to their appropriate tRNAs.

Activation energy (E_a) • The energy barrier that blocks the tendency for a set of chemical substances to react.

Active site • The region on the surface of an enzyme where the substrate binds, and where catalysis occurs.

Active transport • The transport of a substance across a biological membrane against a concentration gradient—that is, from a region of low concentration (of that substance) to a region of high concentration. Active transport requires the expenditure of energy and is a saturable process. (Contrast with facilitated diffusion, free diffusion; see primary active transport, secondary active transport.)

Adaptation (a dap tay' shun) • In evolutionary biology, a particular structure, physiological process, or behavior that makes an organism better able to survive and reproduce. Also, the evolutionary process that leads to the development or persistence of such a trait.

Adenine (a' den een) • A nitrogen-containing base found in nucleic acids, ATP, NAD, etc.

Adenosine triphosphate • See ATP.

Adenylate cyclase • Enzyme catalyzing the formation of cyclic AMP from ATP.

Adrenal (a dree' nal) [L. ad-: toward + renes: kidneys] • An endocrine gland located near the kidneys of vertebrates, consisting of two glandular parts, the cortex and medulla.

Adrenaline • See epinephrine.

Adrenocorticotropin • See ACTH.

Adsorption • Binding of a gas or a solute to the surface of a solid.

Aerobic (air oh' bic) [Gr. aer: air + bios: life] • In the presence of oxygen, or requiring oxygen.

Afferent (af ur unt) [L. ad: to + ferre: to bear] • To or toward, as in a neuron that carries impulses to the central nervous system, or a blood vessel that carries blood to a structure. (Contrast with efferents.)

AIDS (acquired immune deficiency syndrome) • Condition caused by a virus (HIV) in which the body's helper T lymphocytes are reduced, leaving the victim subject to opportunistic diseases.

Aldehyde (al' duh hide) • A compound with a -CHO functional group. Many sugars are aldehydes. (Contrast with ketone.)

Aldosterone (al dahs' ter own) • A steroid hormone produced in the adrenal cortex of mammals. Promotes secretion of potassium and reabsorption of sodium in the kidney.

Alga (al' gah) (plural: algae) [L.: seaweed] • Any one of a wide diversity of protists belonging to the phyla Pyrrophyta, Chrysophyta, Phaeophyta, Pdiophyta, and Chlorophyta.

Allele (a leel') [Gr. alios: other] • The alternate forms of a genetic character found at a given locus on a chromosome.

Allele frequency • The relative proportion of a particular allele in a specific population.

Allergy [Ger. allergie: altered reaction] • An overreaction to an antigen in amounts that do not affect most people; often involves IgE antibodies.

Allometric growth • A pattern of growth in which some parts of the body of an organism grow faster than others, resulting in a change in body proportions as the organism grows.

Allopatric speciation (al' lo pat' rick) [Gr. alios: other + patria: fatherland] • Also called geographical speciation, this is the formation of two species from one when reproductive isolation occurs because of the the interposition of (or crossing of) a physical geographic barrier such as a river. (Contrast with parapatric speciation, sympatric speciation.)

GLOSS \ ^

Allopolyploid • A polyploid in which the chromosome sets are derived from more than one species.

Allosteric regulation (al' lo steer' \) [Gr. alios: other + stereos: structure] • Regulation of the activity of a protein by the binding of an effector molecule at a site other than the active site.

Alpha helix • I\ pe of protein secondary

structure; a right-handed spiral.

Alternation of generations • The succession of haploid and diploid phases in some sexually reproducing organisms, notably plants.

Altruism • A behavior whose performance harms the actor but benefits other individuals.

Alveolus (al ve' o lus) (plural: alveoli) [L. alveus: cavity] • A small, baglike cavity, especially the blind sacs of the lung.

Amensalism (a men' sul ism) • Interaction in which one animal is harmed and the other is unaffected. (Contrast with commensalism, mutualism.)

Amine • An organic compound with an amino group (see Amino acid).

Amino acid • An organic compound of the general formula $H_2N-CH(R)-COOH$, where R can be one of 20 or more different side groups. An amino acid is so named because it has both a basic amine group, $-NH_2$, and an acidic carboxyl group, $-COOH$. Proteins are polymers of amino acids.

Ammonotelic (am moan' o teel' ic) [Gr. telos: end] • Describes an organism in which the final product of breakdown of nitrogen-containing compounds (primarily proteins) is ammonia. (Contrast with ureotelic, uricotelic.)

Amniocentesis • A medical procedure in which cells from the fetus are obtained from the amniotic fluid. The genetic material of the cells is then examined. (Contrast with chorionic villus sampling.)

Amniote • An organism that lays eggs that can be incubated in air (externally) because the embryo is enclosed by a fluid-filled sac. Birds and reptiles are amniotes.

Amphipathic (am' fi path' ic) [Gr. amphi: both + pathos: emotion] • Of a molecule, having both hydrophilic and hydrophobic regions.

Amylase (am' ill ase) • Any of a group of enzymes that digest starch.

Anabolism (an ab' uh liz' em) [Gr. ana: up, throughout + ballein: to throw] • Synthetic reactions of metabolism, in which complex molecules are formed from simpler ones. (Contrast with catabolism.)

Anaerobic (an ur row' bie) [Gr. an: not + aer: air + bios: life] • Occurring without the use of molecular oxygen, O_2 .

Anagenesis • Evolutionary change in a single lineage over time.

Analogy (a nal' o jee) [Gr. analogia: resembling] • A resemblance in function, and often appearance as well, between two structures which is due to convergence in evolution rather than to common ancestry. (Contrast with homology.)

Anaphase (an' a phase) [Gr. ana: indicating upward progress] • The stage in nuclear division at which the first separation of sister chromatids (or, in the first meiotic division, of paired homologues) occurs. Anaphase lasts from the moment of first separation to the time at which the moving chromosomes converge at the poles of the spindle.

Anaphylactic shock • A precipitous drop in blood pressure caused by loss of fluid from capillaries because of an increase in their permeability stimulated by an allergic reaction.

Ancestral trait • Trait shared by a group of organisms as a result of descent from a common ancestor.

Androgens (an' dro jens) • The male sex steroids.

Aneuploidy (an' you ploy dee) • A condition in which one or more chromosomes or pieces of chromosomes are either lacking or present in excess.

Angiosperm (an' jee oh spurm) [Gr. angion: vessel + sperma: seed] • One of the flowering plants; literally, one whose seed is carried in a "vessel," which is the fruit. (See fruit.)

Angiotensin (an' jee oh ten' sin) • A peptide hormone that raises blood pressure by causing peripheral vessels to constrict; maintains glomerular filtration by constricting efferent glomerular vessels; stimulates thirst; and stimulates the release of aldosterone.

Animal [L. animus: breath, soul] • A member of the kingdom Animalia. In general, a multicellular eukaryote that obtains its food by ingestion.

Animal hemisphere • The metabolically active upper portion of some animal eggs, zygotes, and embryos, which does not contain the dense nutrient yolk. The animal pole refers to the very top of the egg or embryo. (Contrast with vegetal hemisphere.)

Anion (an' eye one) • An ion with one or more negative charges. (Contrast with cation.)

Anisogamy (an' eye sog' a mee) [Gr. aniso: unequal + gamos: marriage] • The existence of two dissimilar gametes (egg and sperm).

Annual • Referring to a plant whose life cycle is completed in one growing season. (Contrast with biennial, perennial.)

Anterior pituitary • The portion of the vertebrate pituitary gland that derives from gut epithelium and produces tropic hormones.

Anther (an' thur) [Gr. anthos: flower] • A pollen-bearing portion of the stamen of a flower.

Antheridium (an' thur id' ee um) (plural: antheridia) [Gr. anthems: blooming] • The multicellular structure that produces the sperm in bryophytes and ferns.

Antibody • One of millions of proteins, produced by the immune system, that specifically recognizes a foreign substance and initiates its removal from the body.

Anticodon • A "triplet" of three nucleotides in transfer RNA that is able to pair with a complementary triplet (a codon) in messenger RNA, thus aligning the transfer RNA on the proper place on the messenger. The codon (and, reciprocally, the anticodon) codes for a specific amino acid.

Antidiuretic hormone • A hormone that controls water reabsorption in the mammalian kidney. Also called vasopressin.

Antigen (an' ti jun) • Any substance that stimulates the production of an antibody or antibodies in the body of a vertebrate.

Antigen processing • The breakdown of antigenic proteins into smaller fragments, which are then presented on the cell surface, along with MHC proteins, to T cells.

Antigenic determinant • A specific region of an antigen, which is recognized by and binds to a specific antibody.

Antiport • A membrane transport process that carries one substance in one direction and another in the opposite direction. (Contrast with symport.)

Antisense nucleic acid • A single-stranded RNA or DNA complementary to and thus targeted against the mRNA transcribed from a harmful gene such as an oncogene.

Anus (a' nus) • Opening through which digestive wastes are expelled, located at the posterior end of the gut.

Aorta (a or' tuh) [Gr. aorte: aorta] • The main trunk of the arteries leading to the systemic (as opposed to the pulmonary) circulation.

Apex (a' pecks) • The tip or highest point of a structure, as the apex of a growing stem or root.

Apical (a' pi kul) • Pertaining to the apex, or tip, usually in reference to plants.

Apical dominance • Inhibition by the apical bud of the growth of axillary buds.

Apical meristem • The meristem at the tip of a shoot or root; responsible for the plant's primary growth.

Apomixis (ap oh mix' is) [Gr. apo: away from + mixis: sexual intercourse] • The asexual production of seeds.

Apoplast (ap' oh plast) • In plants, the continuous meshwork of cell walls and extracellular spaces through which material can pass without crossing a plasma membrane. (Contrast with symplast.)

Apoptosis (ay' pu toh sis) • A series of genetically programmed events leading to cell death.

Aquaporin • A transport protein in plant and animals cells through which water passes in osmosis.

Archegonium (ar' ke go' nee um) [Gr. archegonos: first of a kind] • The multicellular structure that produces eggs in bryophytes, ferns, and gymnosperms.

Archenteron (ark en' ter on) [Gr. archos: beginning + enteron: bowel] • The earliest primordial animal digestive tract.

Arteriosclerosis • See atherosclerosis.

GLOSSARY

Artery • A muscular blood vessel carrying oxygenated blood away from the heart to other parts of the body. (Contrast with vein.)

Ascus (ass' cuss) [Gr. askos: bladder] • In fungi belonging to the phylum Ascomycota (the sac fungi), the club-shaped sporangium within which spores (ascospores) are produced by meiosis.

Asexual • Without sex.

Assortative mating • A breeding system in which mates are selected on the basis of a particular trait or group of traits.

Atherosclerosis (ath' er oh sklair oh' sis) • A disease of the lining of the arteries characterized by fatty, cholesterol-rich deposits in the walls of the arteries. When fibroblasts infiltrate these deposits and calcium precipitates in them, the disease become arteriosclerosis, or "hardening of the arteries."

Atmosphere • The gaseous mass surrounding our planet. Also: a unit of pressure, equal to the normal pressure of air at sea level.

Atom [Gr. atomos: indivisible] • The smallest unit of a chemical element. Consists of a nucleus and one or more electrons.

Atomic mass (also called atomic weight) • The average mass of an atom of an element on the amu scale. (The average depends upon the relative amounts of different isotopes of an element on Earth.)

Atomic number • The number of protons in the nucleus of an atom, also equal to the number of electrons around the neutral atom. Determines the chemical properties of the atom.

ATP (adenosine triphosphate) • A compound containing adenine, ribose, and three phosphate groups. When it is formed, useful energy is stored; when it is broken down (to ADP or AMP), energy is released to drive endergonic reactions. ATP is an energy storage compound.

ATP synthase • An integral membrane protein that couples the transport of protons with the formation of ATP.

Atrium (a' tree um) • A body cavity, as in the hearts of vertebrates. The thin-walled chamber(s) entered by blood on its way to the ventricle(s). Also, the outer ear.

Autoimmune disease • A disorder in which the immune system attacks the animal's own antigens.

Autonomic nervous system • The system (which in vertebrates comprises sympathetic and parasympathetic subsystems) that controls such involuntary functions as those of guts and glands.

Autosome • Any chromosome (in a eukary-ote) other than a sex chromosome.

Autotroph (au' tow trow' fik) [Gr. antos: self + trophic: food] • An organism that is capable of living exclusively on inorganic materials, water, and some energy source such as sunlight or chemically reduced matter. (Contrast with heterotroph.)

Auxin (awk' sin) [Gr. auxein: increase] • In plants, a substance (indoleacetic acid) that regulates growth and various aspects of development.

Auxotroph (awks' o trofe) [Gr. auxanein: to grow + trophe: food] • A mutant form of an organism that requires a nutrient or nutrients not required by the wild type, or reference, form of the organism. (Contrast with prototroph.)

Axon [Gr.: axle] • Fiber of a neuron which can carry action potentials. Carries impulses away from the cell body of the neuron; releases a neurotransmitter substance.

Axon hillock • The junction between an axon and its cell body; where action potentials are generated.

Axon terminals • The endings of an axon; they form synapses and release neurotransmitter.

Axoneme (ax' oh neem) • The complex of microtubules and their crossbridges that forms the motile apparatus of a cilium.

Bacillus (buh sil' us) [L.: little rod] • Any of various rod-shaped bacteria.

Bacteriophage (bak teer' ee o fayj) [Gr. bak-terion: little rod + phagein: to eat] • One of a group of viruses that infect bacteria

and ultimately cause their disintegration.

Bacteria (bak teer' ee ah) (singular: bacterium) [Gr. bakterion: little rod] • Prokaryote in the Domain Bacteria. The chromosomes of bacteria are not contained in nuclear envelopes.

Balanced polymorphism [Gr. pohjmorphos: having many forms] • The maintenance of more than one form, or the maintenance at a given locus of more than one allele, at frequencies of greater than one percent in a population. Often results when heterozygotes are superior to both homozygotes.

Bark • All tissues outside the vascular cambium of a plant.

Baroreceptor [Gr. baros: weight] • A pressure-sensing cell or organ.

Barr body • In mammals, an inactivated X chromosome.

Basal body • Centriole found at the base of a eukaryotic flagellum or cilium.

Basal metabolic rate • The minimum rate of energy turnover in an awake (but resting) bird or mammal that is not expending energy for thermoregulation.

Base • (1) A substance which can accept a proton (hydrogen ion; H^+) in solution. (Contrast with acid.) (2) In nucleic acids, a nitrogen-containing molecule that is attached to each sugar in the backbone. (See purine; pyrimidine.)

Base pairing • See complementary base pairing.

Basic • having a pH greater than 7.0 (having a hydrogen ion concentration lower than 10^{-7} molar).

Basidium (bass id' ee yum) • In fungi of the class Basidiomycetes, the characteristic sporangium in which four spores are formed by meiosis and then borne externally before being shed.

Batesian mimicry • Mimicry by a relatively harmless kind of organism of a more dangerous one, by which the mimic enjoys protection from predators that mistake it for

the dangerous model. (Contrast with Mullerian mimicry.)

B cell • A type of lymphocyte involved in the humoral immune response of vertebrates. Upon recognizing an antigenic determinant, a B cell develops into a plasma cell, which secretes an antibody. (Contrast with a T cell.)

Benefit • An improvement in survival and reproductive success resulting from a behavior. (Contrast with cost.)

Benign (be nine') • A tumor that grows to a certain size and then stops, usually with a fibrous capsule surrounding the mass of cells. Benign tumors do not spread (metastasize) to other organs.

Benthic zone [Gr. benthos: bottom of the sea] • The bottom of the ocean. (Contrast with pelagic zone.)

Beta-pleated sheet • Type of protein secondary structure; results from hydrogen bonding between polypeptide regions running antiparallel to each other.

Biennial • Referring to a plant whose life cycle includes vegetative growth in the first year and flowering and senescence in the second year. (Contrast with annual, perennial.)

Bilateral symmetry • The condition in which only the right and left sides of an organism, divided exactly down the back, are mirror images of each other. (Contrast with biradial symmetry)

Bile • A secretion of the liver delivered to the small intestine via the common bile duct. In the intestine, bile emulsifies fats.

Binocular cells • Neurons in the visual cortex that respond to input from both retinas; involved in depth perception.

Binomial (bye nome' ee al) • Consisting of two names; for example, the binomial nomenclature of biology which gives the name of the genus followed by the name of the species.

Biodiversity crisis • The current high rate of loss of species, caused primarily by human activities.

Biogeochemical cycles • Movement of elements through living organisms and the physical environment.

Biogeography • The scientific study of the geographic distribution of organisms.

Biogeographic region • A continental-scale part of Earth that has a biota distinct from that of other such regions.

Biological species concept • The view that a species is most usefully defined as a population or series of populations within which there is a significant amount of gene flow under natural conditions, but which is genetically isolated from other populations.

Bioluminescence • The production of light by biochemical processes in an organism.

Biomass • The total weight of all the living organisms, or some designated group of living organisms, in a given area.

Biome (bye' ome) • A major division of the ecological communities of Earth; characterized by distinctive vegetation.

SSAItt

Biota (bye oh' tah) • All of the organisms including animals, plants, fungi, and microorganisms, found in a given area.

Biotechnology • The use of cells to make medicines, foods and other products useful to humans.

Radial symmetry • Radial symmetry modified so that only two planes can divide the animal into similar halves.

Blastocoel (blass' toe seal) [Br. blastos: sprout + koilos: hollow] • The central, hollow center of a blastula.

Blastodisc (blass' toe disk) • A disk of cells forming on the surface of a large yolk mass, comparable to a blastula, but occurring in animals such as birds and reptiles, in which the mass of yolk restricts cleavage to one side of the egg only.

Blastomere • A cell produced by the division of a fertilized egg.

Blastopore • The opening from the archenteron to the exterior of a gastrula.

Blastula (blass' chu luh) [Gr. blastos: sprout] • An early stage in animal embryology; in many species, a hollow sphere of cells surrounding a central cavity, the blastocoel. (Contrast with blastodisc.)

Blood-brain barrier • A property of the blood vessels of the brain that prevents most chemicals from diffusing from the blood into the brain.

Body plan • A basic structural design that includes an entire animal, its organ systems, and the integrated functioning of its parts. Phylogenetic groups of organisms are classified in part on the basis of a shared body plan.

Bowman's capsule • An elaboration of kidney tubule cells that surrounds a network of capillaries (the glomerulus). Blood is filtered across the walls of these capillaries and the filtrate is collected into Bowman's capsule.

Brain stem • The portion of the vertebrate brain between the spinal cord and the fore-brain.

Brassinosteroids • Plant steroid hormones that promote the elongation of stems and pollen tubes.

Bronchus (plural: bronchi) • The major air-way(s) branching off the trachea into the vertebrate lung.

Brown fat • Fat tissue in mammals that is specialized to produce heat. It has many mitochondria and capillaries, and a protein that uncouples oxidative phosphorylation.

Browser • An animal that feeds on the tissues of woody plants.

Bryophyte (bri' uh fite') [Gr. bruon: moss + phyton: plant] • A moss. Formerly was often used to refer to all the nontracheophyte plants.

Budding • Asexual reproduction in which a more or less complete new organism simply grows from the body of the parent organism and eventually detaches itself.

Buffering • A process by which a system resists change—particularly in pH, in which case added acid or base is partially converted to another form.

C₃ photosynthesis • The form of photosynthesis in which 3-phosphoglycerate is the first stable product, and ribulose biphosphate is the CO₂ receptor.

C₄ photosynthesis • The form of photosynthesis in which oxaloacetate is the first stable product, and phosphoenolpyruvate is the CO₂ acceptor. C₄ plants also perform the reactions of C₃ photosynthesis.

Calcitonin • A hormone produced by the thyroid gland; it lowers blood calcium and promotes bone formation. (Contrast with parathormone.)

Calmodulin (cal mod' joo lin) • A calcium-binding protein found in all animal and plant cells; mediates many calcium-regulated processes.

calorie [L. calor. heat] • The amount of heat required to raise the temperature of one gram of water by one degree Celsius (1°C) from 14.5°C to 15.5°C. In nutrition studies, "Calorie" (spelled with a capital C) refers to the kilocalorie (1 kcal = 1,000 cal).

Calvin-Benson cycle • The stage of photosynthesis in which CO₂ reacts with RuBP to form 3PG, 3PG is reduced to a sugar, and RuBP is regenerated, while other products are released to the rest of the plant.

Calyx (kay' licks) [Gr. kalyx: cup] • All of the sepals of a flower, collectively.

CAM • See crassulacean acid metabolism.

Cambium (kam' bee um) [L. cambiare: to exchange] • A meristem that gives rise to radial rows of cells in stem and root,

increasing them in girth; commonly applied to the vascular cambium which produces wood and phloem, and the cork cambium, which produces bark.

cAMP (cyclic AMP) • A compound, formed from ATP, that mediates the effects of numerous animal hormones. Also needed for the transcription of catabolite-repressible operons in bacteria. Used for communication by cellular slime molds.

Canopy • The leaf-bearing part of a tree. Collectively the aggregate of the leaves and branches of the larger woody plants of an ecological community.

Capillaries [L. capillaris: hair] • Very small tubes, especially the smallest blood-carrying vessels of animals between the termination of the arteries and the beginnings of the veins.

Capsid • The protein coat of a virus.

Carbohydrates • Organic compounds with the general formula $C_n(H_2O)_m$. Common examples are sugars, starch, and cellulose.

Carboxylic acid (kar box sill' ik) • An organic acid containing the carboxyl group, $-COOH$, which dissociates to the carboxylate ion, $-COO^-$.

Carcinogen (car sin' oh jen) • A substance that causes cancer.

Cardiac (kar' dee ak) [Gr. kardia: heart] • Pertaining to the heart and its functions.

Carnivore [L. earn: flesh + vorare: to devour] • An organism that feeds on animal tissue. (Contrast with detritivore, herbivore, omni-vore.)

Carotenoid (ka rah' tuh noid) [L. carota: carrot] • A yellow, orange, or red lipid pigment commonly found as an accessory pigment in photosynthesis; also found in fungi.

Carpel (kar' pel) [Gr. karpos: fruit] • The organ of the flower that contains one or more ovules.

Carrier • (1) In facilitated diffusion, a membrane protein that binds a specific molecule and transports it through the membrane. (2) In respiratory and photosynthetic electron transport, a participating substance such as NAD that exists in both oxidized and reduced forms. (3) In genetics, a person heterozygous for a recessive trait.

Carrying capacity • In ecology, the largest number of organisms of a particular species that can be maintained indefinitely in a given part of the environment.

Cartilage • In vertebrates, a tough connective tissue found in joints, the outer ear, and elsewhere. Forms the entire skeleton in some animal groups.

Casparian strip • A band of cell wall containing suberin and lignin, found in the endodermis. Restricts the movement of water across the endodermis.

Catabolism [Ge. kata: down + ballein: to throw] • Degradational reactions of metabolism, in which complex molecules are broken down. (Contrast with anabolism.)

Catalyst (cat' a list) [Gr. kata-, implying the breaking down of a compound] • A chemical substance that accelerates a reaction without itself being consumed in the overall course of the reaction. Catalysts lower the activation energy of a reaction. Enzymes are biological catalysts.

Cation (cat' eye on) • An ion with one or more positive charges. (Contrast with anion.)

Caudal [L. cauda: tail] • Pertaining to the tail, or to the posterior part of the body.

cDNA • See complementary DNA.

Cecum (see' cum) [L. caecus: blind] • A blind branch off the large intestine. In many nonruminant mammals, the cecum contains a colony of microorganisms that contribute to the digestion of food.

Cell adhesion molecules • Molecules on animal cell surfaces that affect the selective association of cells during development of the embryo.

Cell cycle • The stages through which a cell passes between one division and the next. Includes all stages of interphase and mitosis.

Cell division • The reproduction of a cell to produce two new cells. In eukaryotes, this process involves nuclear division (mitosis) and cytoplasmic division (cytokinesis).

Cell theory • The theory, well established, that organisms consist of cells, and that all cells come from preexisting cells.

Cell wall • A relatively rigid structure that encloses cells of plants, fungi, many protists, and most bacteria. The cell wall gives these cells their shape and limits their expansion in hypotonic media.

GLOSSARY

Cellular immune system • That part of the immune system that is based on the activities of T cells. Directed against parasites, fungi, intracellular viruses, and foreign tissues (grafts). (Contrast with humoral immune system.)

Cellular respiration • See respiration.

Cellulose (sell' you lowss) • A straight-chain polymer of glucose molecules, used by plants as a structural supporting material.

Central dogma • The statement that information flows from DNA to RNA to polypeptide (in retroviruses, there is also information flow from RNA to cDNA).

Central nervous system • That part of the nervous system which is condensed and centrally located, e.g., the brain and spinal cord of vertebrates; the chain of cerebral, thoracic and abdominal ganglia of arthropods.

Centrifuge [L.figere: to flee] • A device in which a sample can be spun around a central axis at high speed, creating a centrifugal force that mimics a very strong gravitational force. Used to separate mixtures of suspended materials.

Centriole (sen' tree ole) • A paired organelle that helps organize the microtubules in animal and protist cells during nuclear division.

Centromere (sen' tro meer) [Gr. centron: center + mews: part] • The region where sister chromatids join.

Centrosome (sen' tro soam) • The major microtubule organizing center of an cell.

Cephalization (sef uh luh zay' shun) [Gr. kephale: head] • The evolutionary trend toward increasing concentration of brain and sensory organs at the anterior end of the animal.

Cerebellum (sair' uh bell' um) [L.: diminutive of cerebrum: brain] • The brain region that controls muscular coordination; located at the anterior end of the hindbrain.

Cerebral cortex • The thin layer of gray matter (neuronal cell bodies) that overlays the cerebrum.

Cerebrum (su ree' brum) [L.: brain] • The dorsal anterior portion of the forebrain, making up the largest part of the brain of mammals. In mammals, the chief coordination center of the nervous system; consists of two cerebral hemispheres.

Cervix (sir' vix) [L.: neck] • The opening of the uterus into the vagina.

cGMP (cyclic guanosine monophosphate)

• An intracellular messenger that is part of signal transmission pathways involving G proteins. (See G protein.)

Channel • A membrane protein that forms an aqueous passageway through which specific solutes may pass by simple diffusion; some channels are gated: they open and close in response to binding of specific molecules.

Chaperone protein • A protein that assists a newly forming protein in adopting its appropriate tertiary structure.

animal

Chemical bond • An attractive force stably linking two atoms.

Chemiosmotic mechanism • The formation of ATP in mitochondria and chloroplasts, resulting from a pumping of protons across a membrane (against a gradient of electrical charge and of pH), followed by the return of the protons through a protein channel with ATPase activity.

Chemoautotroph • An organism that uses carbon dioxide as a carbon source and obtains energy by oxidizing inorganic substances from its environment. (Contrast with chemoheterotroph, photoautotroph, photoheterotroph.)

Chemoheterotroph • An organism that must obtain both carbon and energy from organic substances. (Contrast with chemoautotroph, photoautotroph, photoheterotroph.)

Chemoreceptor • A cell or tissue that senses specific substances in its environment.

Chemosynthesis • Synthesis of food substances, using the oxidation of reduced materials from the environment as a source of energy.

Chiasma (kie az' muh) (plural: chiasmata) [Gr.: cross] • An X-shaped connection between paired homologous chromosomes in prophase I of meiosis. A chiasma is the visible manifestation of crossing over between homologous chromosomes.

Chitin (kye' tin) [Gr. kiton: tunic] • The characteristic tough but flexible organic component of the exoskeleton of arthropods, consisting of a complex, nitrogen-containing polysaccharide. Also found in cell walls of fungi.

Chlorophyll (klor' o fill) [Gr. kloros: green + phyllon: leaf] • Any of a few green pigments associated with chloroplasts or with certain bacterial membranes; responsible for trapping light energy for photosynthesis.

Chloroplast [Gr. kloros: green + plast: a particle] • An organelle bounded by a double membrane containing the enzymes and pigments that perform photosynthesis. Chloroplasts occur only in eukaryotes.

Choanocyte (cho' an oh cite) • The collared, flagellated feeding cells of sponges.

Cholecystokinin (ko' lee sis to kai nin) • A hormone produced and released by the lining of the duodenum when it is stimulated by undigested fats and proteins. It stimulates the gallbladder to release bile and slows stomach activity.

Chorion (kor' ee on) [Gr. kliorkm: afterbirth] • The outermost of the membranes protecting mammal, bird, and reptile embryos; in mammals it forms part of the placenta.

Chorionic villus sampling • A medical procedure that extracts a portion of the chorion from a pregnant woman to enable genetic and biochemical analysis of the embryo. (Contrast with amniocentesis.)

Chromatid (kro' ma tid) • Each of a pair of new sister chromosomes from the time at which the molecular duplication occurs until the time at which the centromeres separate at the anaphase of nuclear division.

Chromatin • The nucleic acid-protein complex found in eukaryotic chromosomes.

Chromatophore (krow mat' o for) [Gr. kroma: color + phoreus: carrier] • A pigment-bearing cell that expands or contracts to change the color of the organism.

Chromosome (krome' o sowm) [Gr. kroma: color + soma: body] • In bacteria and viruses, the DNA molecule that contains most or all of the genetic information of the cell or virus. In eukaryotes, a structure composed of DNA and proteins that bears part of the genetic information of the cell.

Chylomicron (ky low my' cron) • Particles of lipid coated with protein, produced in the gut from dietary fats and secreted into the extracellular fluids.

Chyme (kime) [Gr. kymus, juice] • Created in the stomach; a mixture of ingested food with the digestive juices secreted by the salivary glands and the stomach lining.

Cilium (sil' ee um) (plural: cilia) [L. cilium: eyelash] • Hairlike organelle used for locomotion by many unicellular organisms and for moving water and mucus by many multicellular organisms. Generally shorter than a flagellum.

Circadian rhythm (sir kade' ee an) [L. circa:

approximately + dies: day] • A rhythm in behavior, growth, or some other activity that recurs about every 24 hours under constant conditions.

Circannual rhythm (sir can' you al) [L. circa: approximately + annus: year] • A rhythm of behavior, growth, or some other activity that recurs on a yearly basis.

Citric acid cycle • A set of chemical reactions in cellular respiration, in which acetyl CoA reacts with oxaloacetate to form citric acid, and oxaloacetate is regenerated. Acetyl CoA is oxidized to carbon dioxide, and hydrogen atoms are stored as NADH and FADH₂. Also called the Krebs cycle.

Class • In taxonomy, the category below the phylum and above the order; a group of related, similar orders.

Class I MHC molecules • These cell surface proteins participate in the cellular immune response directed against virus-infected cells.

Class II MHC molecules • These cell surface proteins participate in the cell-cell interactions (of helper T cells, macrophages, and B cells) of the humoral immune response.

Class switching • The process whereby a plasma cell changes the class of immunoglobulin that it synthesizes. This results from the deletion of part of the constant region of DNA, bringing in a new C segment. The variable region is the same as before, so that the new immunoglobulin has the same antigenic specificity.

Clathrin • A fibrous protein on the inner surfaces of animal cell membranes that strengthens coated vesicles and thus participates in receptor-mediated endocytosis.

Clay • A soil constituent comprising particles smaller than 2 micrometers in diameter.

Cleavages • First divisions of the fertilized egg of an animal.

GLOSSAL

Cline • A gradual change in the traits of a species over a geographical gradient.

Cloaca (klo a\ kuh) [L. cloaca: sewer] • In some invertebrates, the posterior part of the gut; in many vertebrates, an opening receiving

material from the digestive reproductive, muscular, excretory systems.

Clonal anergy • When a naive T cell encounters a self-antigen, the T cell may

bind to the antigen but does not receive signals from an antigen-presenting cell. Instead of being activated, the T cell dies (becomes anergic). In this way, we avoid reacting to our own tissue-specific antigens.

Clonal deletion • In immunology, the inactivation or destruction of lymphocyte clones that would produce immune reactions against the animal's own body.

Clonal selection • The mechanism by which exposure to antigen results in the activation of selected T- or B-cell clones, resulting in an immune response.

Clone [Gr. klon: twig, shoot] • Genetically identical cells or organisms produced from a common ancestor by asexual means.

Cnidocytes • The feeding cells of cnidarians, within which nematocysts are housed.

Coacervate (ko as' er vate) [L. coacervare: to heap up] • An aggregate of colloidal particles in suspension.

Coacervate drop • Drops formed when a mixture of large proteins and polysaccharides is shaken in water. The interiors of these drops, which are often very stable, contain most of the proteins and polysaccharides.

Coated vesicle • Vesicle, sometimes formed from a coated pit, with characteristic "bristly" surface; its membrane contains distinctive proteins, including clathrin.

Coccus (kock' us) [Gr. kokkos: berry, pit] • Any of various spherical or spheroidal bacteria.

Cochlea (kock' lee uh) [Gr. kokhlos: a land snail] • A spiral tube in the inner ear of vertebrates; it contains the sensory cells involved in hearing.

Codominance • A condition in which two alleles at a locus produce different phenotypic effects and both effects appear in heterozygotes.

Codon • A "triplet" of three nucleotides in messenger RNA that directs the placement of a particular amino acid into a polypeptide chain. (Contrast with anticodon.)

Coefficient of relatedness • The probability that an allele in one individual is an identical copy, by descent, of an allele in another individual.

Coelom (see' lum) [Gr. koiloma: cavity] • The body cavity of certain animals, which is lined with cells of mesodermal origin.

Coelomate • Having a coelom.

Coenocyte (seen' a sight) [Gr.: common cell] • A "cell" bounded by a single plasma membrane, but containing many nuclei.

Coenzyme • A nonprotein molecule that plays a role in catalysis by an enzyme. The coenzyme may be part of the enzyme molecule or free in solution. Some coenzymes are oxidizing or reducing agents.

Coevolution • Concurrent evolution of two or more species that are mutually affecting each other's evolution.

Cohort (co' hort) [L. cohors: company of soldiers] • A group of similar-age organisms, considered as it passes through time.

Collagen [Gr. kolla: glue] • A fibrous protein found extensively in bone and connective tissue.

Collecting duct • In vertebrates, a tubule that receives urine produced in the nephrons of the kidney and delivers that fluid to the ureter for excretion.

Collenchyma (cull eng' kyma) [Gr. kolla: glue + enchyma: infusion] • A type of plant cell, living at functional maturity, which lends flexible support by virtue of primary cell walls thickened at the corners. (Contrast with parenchyma, sclerenchyma.)

Colon [Gr. kolon: large intestine] • The large intestine.

Commensalism • The form of symbiosis in which one species benefits from the association, while the other is neither harmed nor benefited.

Common bile duct • A single duct that delivers bile from the gallbladder and secretions from the pancreas into the small intestine.

Communication • A signal from one organism (or cell) that alters the pattern of behavior in another organism (or cell) in an adaptive fashion.

Community • Any ecologically integrated group of species of microorganisms, plants, and animals inhabiting a given area.

Companion cell • Specialized cell found adjacent to a sieve tube member in flowering plants.

Comparative analysis • An approach to studying evolution in which hypotheses are tested by measuring the distribution of

states among a large number of species.

Comparative genomics • Computer-aided comparison of DNA sequences between different organisms to reveal genes with related functions.

Compensation point • The light intensity at which the rates of photosynthesis and of cellular respiration are equal.

Competitive inhibitor • A substance, similar in structure to an enzyme's substrate, that binds the active site and thus inhibits a reaction.

Competition • In ecology, use of the same resource by two or more species, when the resource is present in insufficient supply for the combined needs of the species.

Competitive exclusion • A result of competition between species for a limiting resource in which one species completely eliminates the other.

Competitive inhibitor • A substance, similar in structure to an enzyme's substrate, that binds the active site and inhibits a reaction.

Complement system • A group of eleven proteins that play a role in some reactions of the immune system. The complement proteins are not immunoglobulins.

Complementary base pairing • The A-T

(or A-U), T-A (or U-A), C-G and G-C pairing of bases in double-stranded DNA, in transcription, and between tRNA and mRNA.

Complementary DNA (cDNA) • DNA

formed by reverse transcriptase acting with an RNA template; essential intermediate in the reproduction of retroviruses; used as a tool in recombinant DNA technology; lacks introns.

Complete metamorphosis • A change of state during the life cycle of an organism in which the body is almost completely rebuilt to produce an individual with a very different body form. Characteristic of insects such as butterflies, moths, beetles, ants, wasps, and flies.

Compound • (1) A substance made up of atoms of more than one element. (2) Made up of many units, as the compound eyes of arthropods (as opposed to the simple eyes of the same group of organisms).

Condensation reaction • A reaction in which two molecules become connected by a covalent bond and a molecule of water is released. ($AH + BOH \rightarrow AB + H_2O$.)

Cones • (1) In the vertebrate retina: photoreceptors responsible for color vision. (2) In gymnosperms: reproductive structures consisting of many sporophylls packed relatively tightly.

Conidium (ko nid' ee um) [Gr. konis: dust] • An asexual fungus spore borne singly or in chains either apically or laterally on a hypha.

Conifer (kahn' e fer) [Gr. konos: cone + phew: carry] • One of the cone-bearing gymnosperms, mostly trees, such as pines and firs.

Conjugation (kahn' jew gay' shun) [L. con-jugare: yoke together] • The close approximation of two cells during which they exchange genetic material, as in Paramecium and other ciliates, or during which DNA passes from one to the other through a tube, as in bacteria.

Connective tissue • An animal tissue that connects or surrounds other tissues; its cells are embedded in a collagen-containing matrix.

Connexon • In a gap junction, a protein channel linking adjacent animal cells.

Consensus sequences • Short stretches of DNA that appear, with little variation, in many different genes.

Constant region • The constant region in an immunoglobulin is encoded by a single exon and determines the function, but not the specificity, of the molecule. The constant region of the T cell receptor anchors the protein to the plasma membrane.

Constitutive enzyme • An enzyme that is present in approximately constant amounts in a system, whether its substrates are present or absent. (Contrast with inducible enzyme.)

GLOSSARY

Consumer • An organism that eats the tissues of some other organism.

Continental drift • The gradual drifting apart of the world's continents that has occurred over a period of billions of years.

Convergent evolution • The evolution of

similar features independently in unrelated taxa from different ancestral structures.

Cooperative act • Behavior in which two or more individuals interact to their mutual benefit. No conscious awareness by the actors of the effects of their behavior is implied.

Cooption • The act of capturing something for a particular use. In ecology refers to the diversion of ecological production for human use. Such production is said to be coopted.

Copulation • Reproductive behavior that results in a male depositing sperm in the reproductive tract of a female.

Corepressor • A low molecular weight compound that unites with a protein (the repressor) to prevent transcription in a repressible operon.

Cork • A waterproofing tissue in plants, with suberin-containing cell walls. Produced by a cork cambium.

Corolla (ko role' lah) [L.: diminutive of corona: wreath, crown] • All of the petals of a flower, collectively.

Coronary (kor' oh nair ee) • Referring to the blood vessels of the heart.

Corpus luteum (kor' pus loo' tee um) [L. corpus: body + luteum: yellow] A structure formed from a follicle after ovulation; it produces hormones important to the maintenance of pregnancy.

Cortex [L.: bark or rind] • (1) In plants, the tissue between the epidermis and the vascular tissue of a stem or root. (2) In animals, the outer tissue of certain organs, such as the adrenal cortex and cerebral cortex.

Corticosteroids • Steroid hormones produced and released by the cortex of the adrenal gland.

Cost • See energetic cost, opportunity cost, risk cost.

Cotyledon (kot' ul lee' dun) [Gr. kotyledon: a hollow space] • A "seed leaf." An embryonic organ which stores and digests reserve materials; may expand when seed germinates.

Countercurrent exchange • An adaptation that promotes maximum exchange of heat or any diffusible substance between two fluids by the fluids flow in opposite directions through parallel tubes in close approximation to each other. An example is counter-current heat exchange between arterioles and venules in the extremities of some animals.

Covalent bond • A chemical bond that arises from the sharing of electrons between two atoms. Usually a strong bond.

Crassulacean acid metabolism (CAM) • A metabolic pathway enabling the plants that possess it to store carbon dioxide at night and then perform photosynthesis during the day with stomata closed.

Crista (plural; cristae) • A small, shelflike projection of the inner membrane of a mitochondrion; the site of oxidative phosphorylation.

Critical night length • In the photoperiodic flowering response of short-day plants, the length of night above which flowering occurs and below which the plant remains vegetative. (The reverse applies in the case of long-day plants.)

Critical period • The age during which some particular type of learning must take place or during which it occurs much more easily than at other times. Typical of song learning among birds.

Cross section (also called a transverse section) • A section taken perpendicular to the longest axis of a structure.

Crossing over • The mechanism by which linked markers undergo recombination. In general, the term refers to the reciprocal exchange of corresponding segments between two homologous chromatids.

CRP • The cAMP receptor protein that interacts with the promoter to enhance transcription; a lowered cAMP concentration results in catabolite repression.

Crustacean (crus tay' see an) • A member of the phylum Crustacea, such as a crab, shrimp, or sowbug.

Cryptic appearance [Gr. kryptos: hidden] • The resemblance of an animal to some part of its environment, which helps it to escape detection by predators.

Cryptochromes [Gr. kryptos: hidden + kroma: color] • Photoreceptors mediating some blue-light effects in plants and animals.

Culture • (1) A laboratory association of organisms under controlled conditions. (2) The collection of knowledge, tools, values, and rules that characterize a human society.

Cuticle • A waxy layer on the outer surface of a plant or an insect, tending to retard water loss.

Cyanobacteria (sigh an' o bacteria) [Gr. kuanos: the color blue] • A division of photo-synthetic bacteria, formerly referred to as blue-green algae; they lack sexual reproduction, and they use chlorophyll a in their photosynthesis.

Cyclic AMP • See cAMP

Cyclins • Proteins that activate cyclin-dependent kinases, bringing about transitions in the cell cycle.

Cyclin-dependent kinase (cdk) • A kinase is an enzyme that catalyzes the addition of phosphate groups from ATP to target molecules. Cdk's target proteins involved in transitions in the cell cycle and are active only when complexed to additional protein subunits, cyclins.

Cyst (sist) [Gr. kystis: pouch] • (1) A resistant, thick-walled cell formed by some protozoists and other organisms. (2) An abnormal sac, containing a liquid or semisolid substance, produced in response to injury or illness.

Cytochromes (sy' toe chromes) [Gr. kytos: container + chroma: color] • Iron-containing red proteins, components of the electron-

transfer chains in photophosphorylation and respiration.

Cytokinesis (sy' toe kine ee' sis) [Gr. kytos: container + kinein: to move] • The division of the cytoplasm of a dividing cell. (Contrast with mitosis.)

Cytokinin (sy' toe kine' in) [Gr. kytos: container + kinein: to move] • A member of a class of plant growth substances playing roles in senescence, cell division, and other phenomena.

Cytoplasm • The contents of the cell, excluding the nucleus.

Cytoplasmic determinants • In animal development, gene products whose spatial distribution may determine such things as embryonic axes.

Cytosine (site' oh seen) • A nitrogen-containing base found in DNA and RNA.

Cytoskeleton • The network of microtubules and microfilaments that gives a eukaryotic cell its shape and its capacity to arrange its organelles and to move.

Cytosol • The fluid portion of the cytoplasm, excluding organelles and other solids.

Cytotoxic T cells • Cells of the cellular immune system that recognize and directly eliminate virus-infected cells. (Contrast with helper T cells, suppressor T cells.)

Decomposer • See detritivore.

Degeneracy • The situation in which a single amino acid may be represented by any of two or more different codons in messenger RNA. Most of the amino acids can be represented by more than one codon.

Degradative succession • Ecological succession occurring on the dead remains of the bodies of plants and animals, as when leaves or animal bodies rot.

Deletion (genetic) • A mutation resulting from the loss of a continuous segment of a gene or chromosome. Such mutations never revert to wild type. (Contrast with duplication, point mutation.)

Deme (deem) [Gr. demos: common people] • Any local population of individuals belonging to the same species that interbreed with one another.

Demographic processes • The events—such as births, deaths, immigration, and emigration—that determine the number of individuals in a population.

Demographic stochasticity • Random variations in the factors influencing the size, density, and distribution of a population.

Demography • The study of dynamical changes in the sizes, densities, and distributions of populations.

Denaturation • Loss of activity of an enzyme or nucleic acid molecule as a result of structural changes induced by heat or other means.

Dendrite [Gr. dendron: a tree] • A fiber of a neuron which often cannot carry action potentials. Usually much branched and relatively short compared with the axon, and commonly carries information to the cell body of the neuron.

SSAR\

Denitrification • Metabolic activity by which inorganic nitrogen-containing ions are reduced to form nitrogen gas and other products; carried on in certain soil bacteria.

Density dependence • Change in the severity

of action of agents affecting birth and death rate within populations that are directly or inversely related to population density.

Density independence • The state where the severity of action of agents affecting birth and death rates within a population does not change with the density of the population.

Deoxyribonucleic acid • See DNA.

Depolarization • A change in the electric potential across a membrane from a condition in which the inside of the cell is more negative than the outside to a condition in which the inside is less negative, or even positive, with reference to the outside of the cell. (Contrast with hyperpolarization.)

Derived trait • A trait found among members of a lineage that was not present in the ancestors of that lineage.

Dermal tissue system • The outer covering of a plant, consisting of epidermis in the young plant and periderm in a plant with extensive secondary growth. (Contrast with ground tissue system and vascular tissue SJ stem.)

Desmosome (dez' mo sovvm) [Gr. desmos: bond + soma: body] • An adhering junction between animal cells.

Determination • Process whereby an embryonic cell or group of cells becomes fixed into a predictable developmental pathway.

Detritivore (di try' ti vore) [L. detritus: worn away + vorare: to devour] • An organism that obtains its energy from the dead bodies and/or waste products of other organisms.

Deuterostome • A major evolutionary lineage in animals, characterized by radial cleavage, enterocoelous development, and other traits. (Compare with protostome.)

Development • Progressive change, as in structure or metabolism; in most kinds of organisms, development continues throughout the life of the organism.

Diaphragm (dye' uh fram) [Gr. diaphrassein, to barricade] • (1) A sheet of muscle that separates the thoracic and abdominal cavities in mammals; responsible for the action of breathing. (2) A method of birth control in which a sheet of rubber is fitted over the woman's cervix, blocking the entry of sperm.

Diastole (dye ahs' toll ee) [Gr.: dilation] • The portion of the cardiac cycle when the heart muscle relaxes. (Contrast with systole.)

Dicot (short for dicotyledon) [Gr. di: two + kotyledon: a hollow space] • This term, not used in this book, formerly referred to all angiosperms other than the monocots. (See eudicot, monocot.)

Differentiation • Process whereby original-Is- similar cells follow different developmental pathways. The actual expression of determination.

Diffusion • Random movement of molecules or other particles, resulting in even distribution of the particles when no barriers are present.

Digestibility-reducing chemicals •

Defensive chemicals produced by plants that make the plant's tissues difficult to digest.

Digestion • Enzyme-catalyzed process by which large, usually insoluble, molecules (foods) are hydrolyzed to form smaller molecules of soluble substances.

Dihybrid cross • A mating in which the parents differ with respect to the alleles of two loci of interest.

Dikaryon (di care' ee ahn) [Gr. dis: two + karyon: kernel] • A cell or organism carrying two genetically distinguishable nuclei. Common in fungi.

Dioecious (die eesh' us) [Gr.: two houses] • Organisms in which the two sexes are "housed" in two different individuals, so that eggs and sperm are not produced in the same individuals. Examples: humans, fruit flies, oak trees, date palms. (Contrast with monoecious.)

Diploblastic • Having two cell layers. (Contrast with triploblastic.)

Diploid (dip' loid) [Gr. diploos: double] • Having a chromosome complement consisting of two copies (homologues) of each chromosome. A diploid individual (or cell) usually arises as a result of the fusion of two gametes, each with just one copy of each chromosome. Thus, the two homologues in each chromosome pair in a diploid cell are of separate origin, one derived from the female parent and one from the male parent.

Directional selection • Selection in which phenotypes at one extreme of the population distribution are favored. (Contrast with disruptive selection; stabilizing selection.)

Disaccharide • A carbohydrate made up of two monosaccharides (simple sugars).

Dispersal stage • Stage in its life history at which an organism moves from its birthplace to where it will live as an adult.

Displacement activity • Apparently irrelevant behavior performed by an animal under conflict situations, especially when tendencies to attack and escape are closely balanced.

Display • A behavior that has evolved to influence the actions of other individuals.

Disruptive selection • Selection in which phenotypes at both extremes of the population distribution are favored. (Contrast with directional selection; stabilizing selection.)

Distal • Away from the point of attachment or other reference point. (Contrast with proximal.)

Disturbance • A short-term event that disrupts populations, communities, or ecosystems by changing the environment.

Diverticulum (di ver tic' u lum) [L. divertere: turn away] • A small cavity or tube that connects to a major cavity or tube.

Division • A term used by some microbiologists and formerly by botanists, corresponding to the term phylum.

DNA (deoxyribonucleic acid) • The fundamental hereditary material of all living organisms. In eukaryotes, stored primarily in the cell nucleus. A nucleic acid using deoxyribose rather than ribose.

DNA chip • A small glass or plastic square onto which thousands of single-stranded DNA sequences are fixed. Hybridization of cell-derived RNA or DNA to the target sequences can be performed. (See DNA hybridization.)

DNA hybridization • A process by which DNAs from two species are mixed and heated so that interspecific double helices are formed.

DNA ligase • Enzyme that unites Okazaki fragments of the lagging strand during DNA replication; also mends breaks in DNA strands. It connects pieces of a DNA strand and is used in recombinant DNA technology.

DNA methylation • Addition of methyl groups to DNA; plays role in regulation of gene expression; protects a bacterium's DNA against its restriction endonucleases.

DNA polymerase • Any of a group of enzymes that catalyze the formation of DNA strands from a DNA template.

Domain • The largest unit in the current taxonomic nomenclature. Members of the three domains (Bacteria, Archaea, and Eukarya) are believed to have been evolving independently of each other for at least a billion years.

Dominance • In genetic terminology, the ability of one allelic form of a gene to determine the phenotype of a heterozygous individual, in which the homologous chromosome carries both it and a different allele. For example, if A and a are two allelic forms of a gene, A is said to be dominant to a if AA diploids and Aa diploids are phenotypically identical and are distinguishable from aa diploids. The a allele is said to be recessive.

Dominance hierarchy • In animal behavior, the set of relationships within a group of animals, usually established and maintained by aggression, in which one individual has precedence over all others in eating, mating, and other activities.

Dormancy • A condition in which normal activity is suspended, as in some seeds and buds.

Dorsal [L. dorsum: back] • Pertaining to the back or upper surface. (Contrast with ventral.)

Double fertilization • Process virtually unique to angiosperms in which one sperm nucleus combines with the egg to produce a zygote, and the other sperm nucleus combines with the two polar nuclei to produce the first cell of the triploid endosperm.

Double helix • Of DNA: molecular structure in which two complementary polynucleotide strands, antiparallel to each other, form a right-handed spiral.

Duodenum (doo' uh dee' num.) • The beginning portion of the vertebrate small intestine. (Contrast with ileum, jejunum.)

Duplication (genetic) • A mutation resulting from the introduction into the genome

GLOSSARY

of an extra copy of a segment of a gene or chromosome. (Contrast with deletion, point mutation.)

Dynein [Gr. dunamis: power] • A protein that undergoes conformational changes and thus plays a part in the movement of eukaryotic flagella and cilia.

Ecdysone (eck die' sone) [Gr. ek: out of + dyo: to clothe] • In insects, a hormone that induces molting.

Ecological biogeography • The study of the distributions of organisms from an ecological perspective, usually concentrating on migration, dispersal, and species interactions.

Ecological community • The species living together at a particular site.

Ecological niche (nitch) [L. nidus: nest] • The functioning of a species in relation to other species and its physical environment.

Ecological succession • The sequential replacement of one population assemblage by another in a habitat following some disturbance. Succession sometimes ends in a relatively stable ecosystem.

Ecology [Gr. oikos: house + logos: discourse, study] • The scientific study of the interaction of organisms with their environment, including both the physical environment and the other organisms that live in it.

Ecoregion • A large geographic unit characterized by a typical climate and a widespread assemblage of similar species.

Ecosystem (eek' oh sis turn) • The organisms of a particular habitat, such as a pond or forest, together with the physical environment in which they live.

Ecto- (eck' toh) [Gr.: outer, outside] • A prefix used to designate a structure on the outer surface of the body. For example, ectoderm. (Contrast with endo- and meso-.)

Ectoderm [Gr. ektos: outside + derma: skin] • The outermost of the three embryonic tissue layers first delineated during gastrulation. Gives rise to the skin, sense organs, nervous system, etc.

Ectotherm [Gr. ektos: outside + thermos: heat] • An animal unable to control its body temperature. (Contrast with endotherm.)

Edema (i dee' mah) [Gr. oidema: swelling] • Tissue swelling caused by the accumulation of fluid.

Edge effect • The changes in ecological processes in a community caused by physical and biological factors originating in an adjacent community.

Effector • Any organ, cell, or organelle that moves the organism through the environment or else alters the environment to the organism's advantage. Examples include muscle, bone, and a wide variety of exocrine glands.

Effector cell • A lymphocyte that performs a role in the immune system without further differentiation.

Effector phase • In this phase of the immune response, effector T cells called cytotoxic T cells attack virus-infected cells, and effector helper T cells assist B cells to

differentiate into plasma cells, which release antibodies.

Efferent [L. ex: out + fare: to bear] • Away from, as in neurons that conduct action potentials out from the central nervous system, or arterioles that conduct blood away from a structure. (Contrast with afferent.)

Egg • In all sexually reproducing organisms, the female gamete; in birds, reptiles, and some other vertebrates, a structure within which early embryonic development occurs.

Elasticity • The property of returning quickly to a former state after a disturbance.

Electrocardiogram (EKG) • A graphic recording of electrical potentials from the heart.

Electroencephalogram (EEG) • A graphic recording of electrical potentials from the brain.

Electromyogram (EMG) • A graphic recording of electrical potentials from muscle.

Electron (e lek' tron) [L. electrum: amber (associated with static electricity), from Gr. slektor: bright sun (color of amber)] • One of the three most important fundamental particles of matter, with mass approximately 0.00055 amu and charge -1.

Electronegativity • The tendency of an atom to attract electrons when it occurs as part of a compound.

Electrophoresis (e lek' tro fo ree' sis) [L. electrum: amber + Gr. phorein: to bear] • A separation technique in which substances are separated from one another on the basis of their electric charges and molecular weights.

Electrotonic potential • In neurons, a hyperpolarization or small depolarization of the membrane potential induced by the application of a small electric current. (Contrast with action potential, resting potential.)

Elemental substance • A substance composed of only one type of atom.

Embolus (em' buh lus) [Gr. embolos: inserted object; stopper] • A circulating blood clot. Blockage of a blood vessel by an embolus or by a bubble of gas is referred to as an embolism. (Contrast with thrombus.)

Embryo [Gr. en-: in + bryein: to grow] • A young animal, or young plant sporophyte, while it is still contained within a protective structure such as a seed, egg, or uterus.

Embryo sac • In angiosperms, the female gametophyte. Found within the ovule, it consists of eight or fewer cells, membrane bounded, but without cellulose walls between them.

Emergent property • A property of a complex system that is not exhibited by its individual component parts.

Emigration • The deliberate and usually oriented departure of an organism from the habitat in which it has been living.

3' End (3-prime) • The end of a DNA or RNA strand that has a free hydroxyl group at the 3'-carbon of the sugar (deoxyribose or ribose).

5' End (5-prime) • The end of a DNA or RNA strand that has a free phosphate group at the 5'-carbon of the sugar (deoxyribose or ribose).

Endemic (en dem' ik) [Gr. endemos: dwelling in a place] • Confined to a particular region, thus often having a comparatively restricted distribution. •

Endergonic reaction • One for which energy must be supplied. (Contrast with exergonic reaction.)

Endo- [Gr.: within, inside] • A prefix used to designate an innermost structure. For example, endoderm, endocrine. (Contrast with ecto-, meso-.)

Endocrine gland (en' doh krin) [Gr. eudon: inside + krincin: to separate] • Any gland, such as the adrenal or pituitary gland of vertebrates, that secretes certain substances, especially hormones, into the body through the blood.

Endocrinology • The study of hormones and their actions.

Endocytosis • A process by which liquids or solid particles are taken up by a cell through invagination of the plasma membrane. (Contrast with exocytosis.)

Endoderm [Gr. endow, within + derma: skin] • The innermost of the three embryonic tissue layers first delineated during gastrulation. Gives rise to the digestive and respiratory tracts and structures associated with them.

Endodermis [Gr. eudon: within + derma: skin] • In plants, a specialized cell layer marking the inside of the cortex in roots and some stems. Frequently a barrier to free diffusion of solutes.

Endomembrane system • Endoplasmic reticulum plus Golgi apparatus plus, when present, lysosomes; thus, a system of membranes that exchange material with one another.

Endoplasmic reticulum [Gr. endon: within + L. plasma: form; L. reticulum: little net] • A system of membrane-bounded tubes and flattened sacs found in the cytoplasm of eukaryotes. Exists as rough ER, studded with ribosomes; and smooth ER, lacking ribosomes.

Endorphins • Naturally occurring, opiatelike substances in the mammalian brain.

Endoskeleton [Gr. endon: within + skleros: hard] • A skeleton covered by other, soft body tissues. (Contrast with exoskeleton.)

Endosperm [Gr. endon: within + spernia: seed] • A specialized triploid seed tissue found only in angiosperms; contains stored food for the developing embryo.

Endosymbiosis [Gr. endon: within + syn: together + bios: life] • The living together of two species, with one living inside the body (or even the cells) of the other.

Endosymbiotic theory • Theory that the eukaryotic cell evolved from a prokaryote that contained other, endosymbiotic prokaryotes.

Endotherm [Gr. endon: within + thermos: hot] • An animal that can control its body temperature by the expenditure of its own

SSAR\

metabolic energy. (Contrast with ectotherm.)

Endotoxins [Gr. endon: within + I. toxicum: poison] • Lipopolysaccharides released by the lysis of some Gram-negative bacteria that cause fever and vomiting in a host organism.

Energetic cost • [the difference between the energy an animal would have expended had it rested, and that expended in performing a behavior.]

Energy • The capacity to do work.

Enhancer • In eukaryotes, a DNA sequence, lying on either side of the gene it regulates, that stimulates a specific promoter.

Enterocoelous development • A pattern of development in which the coelum is formed by an outpocketing of the embryonic gut (enteron).

Enterokinase (ent uh row kine' ase) • An enzyme secreted by the mucosa of the duodenum. It activates the zymogen trypsinogen to create the active digestive enzyme trypsin.

Entrainment • With respect to circadian rhythms, the process whereby the period is adjusted to match the 24-hour environmental cycle.

Entropy (en' tro pee) [Gr. en: in + tropein: to change] • A measure of the degree of disorder in any system. A perfectly ordered system has zero entropy; increasing disorder is measured by positive entropy. Spontaneous reactions in a closed system are always accompanied by an increase in disorder and entropy.

Environment • An organism's surroundings, both living and nonliving; includes temperature, light intensity, and all other

species that influence the focal organism.

Environmental toxicology • The study of the distribution and effects of toxic compounds in the environment.

Enzyme (en' zime) [Gr. en: in + zyme: yeast] • A protein, on the surface of which are chemical groups so arranged as to make the enzyme a catalyst for a chemical reaction.

Epi- [Gr.: upon, over] • A prefix used to designate a structure located on top of another; for example: epidermis, epiphyte.

Epicotyl (epp' i kot' il) [Gr. epi: upon + kotyle: something hollow] • That part of a plant embryo or seedling that is above the cotyledons.

Epidermis [Gr. epi: upon + derma: skin] • In plants and animals, the outermost cell layers. (Only one cell layer thick in plants.)

Epididymis (epuh did' uh mus) [Gr. epi: upon + didymos: testicle] • Coiled tubules in the testes that store sperm and conduct sperm from the seminiferous tubules to the vas deferens.

Epinephrine (ep i nef rin) [Gr. epi: upon + nephros: a kidney] • The "fight or flight" hormone. Produced by the medulla of the adrenal gland, it also functions as a neurotransmitter. Also known as adrenaline.

Epiphyte (ep' e fyte) [Gr. epi: upon + phyton: plant] • A specialized plant that grows on

the surface of other plants but does not parasitize them.

Episome • A plasmid that may exist either free or integrated into a chromosome. (See plasmid.)

Epistasis • An interaction between genes, in which the presence of a particular allele of one gene determines whether another gene will be expressed.

Epithelium • In animals, a layer of cells covering or lining an external surface or a cavity.

Equilibrium • (1) In biochemistry, a state in which forward and reverse reactions are proceeding at counterbalancing rates, so there is no observable change in the concentrations of reactants and products. (2) In evolutionary genetics, a condition in which allele and genotype frequencies in a population are constant from generation to generation. .

Erythrocyte (ur rith' row sight) [Gr. erythros: red + kytos: hollow vessel] • A red blood cell.

Esophagus (i soff i gus) [Gr. oisophagos: gullet] • That part of the gut between the pharynx and the stomach.

Ester linkage • A condensation (water-releasing) reaction in which the carboxyl group of a fatty acid reacts with the hydroxyl group of an alcohol. Lipids are formed in this way.

Estivation (ess tuh vay' shun) [L. aestivalis: summer] • A state of dormancy and hypometabolism that occurs during the summer; usually a means of surviving drought and/or intense heat. Contrast with hibernation.

Estrogen • Any of several steroid sex hormones, produced chiefly by the ovaries in mammals.

Estrus (es' truss) [L. oestrus: frenzy] • The period of heat, or maximum sexual receptivity, in some female mammals. Ordinarily, the estrus is also the time of release of eggs in the female.

Ethylene • One of the plant hormones, the gas $H_2C=CH_2$.

Euchromatin • Chromatin that is diffuse and non-staining during interphase; may be transcribed. (Contrast with heterochromatin.)

Eudicots (yew di' kots) [Gr. eu: true + di: two + kotyledon: a cup-shaped hollow] • Members of the angiosperm class Eudicotyledones, flowering plants in which the embryo produces two cotyledons prior to germination. Leaves of most eudicots have major veins arranged in a branched or reticulate pattern.

Eukaryotes (yew car' ry otes) [Gr. eu: true + karyon: kernel or nucleus] • Organisms whose cells contain their genetic material inside a nucleus. Includes all life other than the viruses, Archaeobacteria, and Eubacteria.

Eusocial • Term applied to insects, such as termites, ants, and many bees and wasps, in which individuals cooperate in the care of offspring, there are sterile castes, and generations overlap.

Eutrophication (yoo trofe' ik ay' shun) [Gr. eu-: well + trephein: to flourish] • The addition of nutrient materials to a body of water, resulting in changes to species composition therein.

Evolution • Any gradual change. Organic evolution, often referred to as evolution, is any genetic and resulting phenotypic change in organisms from generation to generation.

Evolutionary agent • Any factor that influences the direction and rate of evolutionary changes.

Evolutionarily conservative • Traits of organisms that evolve very slowly.

Evolutionary innovations • Major changes in body plans of organisms; these have been very rare during evolutionary history.

Evolutionary radiation • The proliferation of species within a single evolutionary lineage.

Evolutionary reversal • The reappearance of the ancestral state of a trait in a lineage in which that trait had acquired a derived state.

Excision repair • The removal and damaged DNA and its replacement by the appropriate nucleotides.

Excitatory postsynaptic potential (EPSP) •

A change in the resting potential of a postsynaptic membrane in a positive (depolarizing) direction. (Contrast with inhibitory postsynaptic potential.)

Excretion • Release of metabolic wastes by an organism.

Exergonic reaction • A reaction in which free energy is released. (Contrast with endergonic reaction.)

Exo- (eks' oh) • Same as ecto-.

Exocrine gland (eks' oh krin) [Gr. exo: outside + krinein: to separate] • Any gland, such as a salivary gland, that secretes to the outside of the body or into the gut.

Exocytosis • A process by which a vesicle within a cell fuses with the plasma membrane and releases its contents to the outside. (Contrast with endocytosis.)

Exon • A portion of a DNA molecule, in eukaryotes, that codes for part of a polypeptide. (Contrast with intron.)

Exoskeleton (eks' oh skel' e ton) [Gr. exos: outside + skleros: hard] • A hard covering on the outside of the body to which muscles are attached. (Contrast with endoskeleton.)

Exotoxins • Highly toxic proteins released by living, multiplying bacteria.

Experiment • A scientific method in which particular factors are manipulated while other factors are held constant so that the potential influences of the manipulated factors can be determined.

Exponential growth • Growth, especially in the number of organisms in a population, which is a simple function of the size of the growing entity: the larger the entity, the faster it grows. (Contrast with logistic growth.)

Expression vector • A DNA vector, such as a plasmid, that carries a DNA sequence that

^

GLOSSARY

includes the adjacent sequences for its expression into mRNA and protein in a host cell.

Expressivity • The degree to which a genotype is expressed in the phenotype— may be affected by the environment.

Extensor • A muscle the extends an appendage.

Extinction • The termination of a lineage of organisms.

Extrinsic protein • A membrane protein found only on the surface of the membrane. (Contrast with intrinsic protein.)

F₁ generation • The immediate progeny of a parental (P) mating; the first filial generation.

F₂ generation • The immediate progeny of a mating between members of the F₁ generation.

Facilitated diffusion • Passive movement through a membrane involving a specific carrier protein; does not proceed against a concentration gradient. (Contrast with active transport, free diffusion.)

Family • In taxonomy, the category below the order and above the genus; a group of related, similar genera.

Fat • A triglyceride that is solid at room temperature. (Contrast with oil.)

Fatty acid • A molecule with a long hydrocarbon tail and a carboxyl group at the other end. Found in many lipids.

Fauna (faw' nah) • All of the animals found in a given area. (Contrast with flora.)

Feces [L. faeces: dregs] • Waste excreted from the digestive system.

Feedback control • Control of a particular step of a multistep process, induced by the presence or absence of a product of one of the later steps. A thermostat regulating the flow of heating oil to a furnace in a home is a negative feedback control device.

Fermentation (fur men tay' shun) [L. fcr-mentum: yeast] • The degradation of a substance such as glucose to smaller

molecules with the extraction of energy, without the use of oxygen (i.e., anaerobically). Involves the glycolytic pathway.

Fertilization • Union of gametes. Also known as syngamy

Fertilization membrane • A membrane surrounding an animal egg which becomes rapidly raised above the egg surface within seconds after fertilization, serving to prevent entry of a second sperm.

Fetus • The latter stages of an embryo that is still contained in an egg or uterus; in humans, the unborn young from the eighth week of pregnancy to the moment of birth.

Fiber • An elongated and tapering cell of flowering plants, usually with a thick cell wall. Serves a support function.

Fibrin • A protein that polymerizes to form long threads that provide structure to a blood clot.

Filter feeder • An organism that feeds upon much smaller organisms, that are suspended in water or air, by means of a straining device.

Filtration • In the excretory physiology of some animals, the process by which the initial urine is formed; water and most solutes are transferred into the excretory tract, while proteins are retained in the blood or hemolymph.

First law of thermodynamics • Energy can be neither created nor destroyed.

Fission • Reproduction of a prokaryote by division of a cell into two comparable progeny cells.

Fitness • The contribution of a genotype or phenotype to the composition of subsequent generations, relative to the contribution of other genotypes or phenotypes. (See inclusive fitness.)

Fixed action pattern • A behavior that is genetically programmed.

Flagellum (fla jell' um) (plural: flagella) [L. flagellum: whip] • Long, whiplike appendage that propels cells. Prokaryotic flagella differ sharply from those found in eukaryotes.

Flexor • A muscle that flexes an appendage.

Flora (flore' ah) • All of the plants found in a given area. (Contrast with fauna.)

Florigen • A plant hormone (not yet isolated) involved in the conversion of a vegetative shoot apex to a flower.

Flower • The total reproductive structure of an angiosperm; its basic parts include the calyx, corolla, stamens, and carpels.

Fluorescence • The emission of a photon of visible light by an excited atom or molecule.

Follicle [L.folliculiis: little bag] • In female mammals, an immature egg surrounded by nutritive cells.

Follicle-stimulating hormone • A

gonadotropic hormone produced by the anterior pituitary

Food chain • A portion of a food web, most commonly a simple sequence of prey species and the predators that consume them.

Food web • The complete set of food links between species in a community; a diagram indicating which ones are the eaters and which are consumed.

Forb • Any broad-leaved (dicotyledonous), herbaceous plant. Especially applied to such plants growing in grasslands.

Fossil • Any recognizable structure originating from an organism, or any impression from such a structure, that has been preserved over geological time.

Fossil fuel • A fuel (particularly petroleum products) composed of the remains of organisms that lived in the remote past.

Founder effect • Random changes in allele frequencies resulting from establishment of a population by a very small number of individuals.

Fovea [L. fovea; a small pit] • The area, in the vertebrate retina, of most distinct vision.

Frame-shift mutation • A mutation resulting from the addition or deletion of a single base pair in the DNA sequence of a gene. As

a result of this, mRNA transcribed from such a gene is translated normally until the ribosome reaches the point at which the mutation has occurred. From that point on, codons are read out of proper register and the amino acid sequence bears no resemblance to the normal sequence. (Contrast with missense mutation, nonsense mutation, synonymous mutation.)

Free energy • That energy which is available for doing useful work, after allowance has been made for the increase or decrease of disorder. Designated by the symbol G (for Gibbs free energy), and defined by: $G = H - TS$, where H = heat, S =

entropy, and T = absolute (Kelvin) temperature.

Frequency-dependent selection • Selection that changes in intensity with the proportion of individuals having the trait.

Fruit • In angiosperms, a ripened and mature ovary (or group of ovaries) containing the seeds. Sometimes applied to reproductive structures of other groups of plants, and includes any adjacent parts which may be fused with the reproductive structures.

Fruiting body • A structure that bears spores.

Fundamental niche • The range of condition under which an organism could survive if it were the only one in the environment. (Contrast with realized niche.)

Fungus (fung' gus) • A member of the kingdom Fungi, a (usually) multicellular eukaryote with absorptive nutrition.

G₁ phase • In the cell cycle, the gap between the end of mitosis and the onset of the S phase.

G₂ phase • In the cell cycle, the gap between the S (synthesis) phase and the onset of mitosis.

G protein • A membrane protein involved in signal transduction; characterized by binding guanyl nucleotides. The activation of certain receptors activates the G protein, which in turn activates adenylate cyclase. G protein activation involves binding a GTP molecule in place of a GDP molecule.

Gametangium (gam i tan' gee um) [Gr. gamos: marriage + angeion: vessel or reservoir] • Any plant or fungal structure within which a gamete is formed.

Gamete (gam' eet) [Gr. gamete: wife, gametes: husband] • The mature sexual reproductive cell: the egg or the sperm.

Gametocyte (ga meet' oh site) [Gr. gamete: wife, gametes: husband + kytos: cell] • The cell that gives rise to sex cells, either the eggs or the sperm. (See oocyte and spermatocyte.)

Gametogenesis (ga meet' oh jen' e sis) [Gr. gamete: wife, gametes: husband + genesis: source] • The specialized series of cellular divisions that leads to the production of sex cells (gametes). (Contrast with oogenesis and spermatogenesis.)

Gametophyte (ga meet' oh fyte) • In plants and photosynthetic protists with alternation of generations, the haploid phase that produces the gametes. (Contrast with sporo-phyte.)

^s \KN

Ganglion (gang* glee un) [Gr.: tumor] • \ group or concentration of neuron cell bodies.

Gap junction • A 2-7-nanometer gap between plasma membranes of two animal cells, spanned by protein channels. Gap junctions allow chemical substances or electrical signals to pass from cell to cell.

Gas exchange • In animals, the process of taking up oxygen from the environment and releasing carbon dioxide to the environment.

Gastrovascular cavity • Serving for both digestion (gastro) and circulation (vascular); in particular, the central cavity of the body of jellyfish and other cnidarians.

Gastrula (gas' true luh) [Gr. gaster. stomach]

• An embryo forming the characteristic three cell layers (ectoderm, endoderm, and mesoderm) which will give rise to all of the major tissue systems of the adult animal.

Gastrulation • Development of a blastula into a gastrula.

Gated channel • A channel (membrane protein) that opens and closes in response to binding of specific molecules or to changes in membrane potential.

Gel electrophoresis (jel ul lee tro for' eesis)

• A semisolid matrix suspended in a salty buffer in which molecules can be separated on the basis of their size and charge when current is passed through the gel.

Gene [Gr. gen: to produce] • A unit of heredity. Used here as the unit of genetic function which carries the information for a single polypeptide.

Gene amplification • Creation of multiple copies of a particular gene, allowing the production of large amounts of the RNA transcript (as in rRNA synthesis in oocytes).

Gene cloning • Formation of a clone of bacteria or yeast cells containing a particular foreign gene.

Gene family • A set of identical, or once-identical, genes, derived from a single parent gene; need not be on the same chromosomes; classic example is the globin family in vertebrates.

Gene flow • The exchange of genes between different species (an extreme case referred to as hybridization) or between different populations of the same species caused by migration following breeding.

Gene pool • All of the genes in a population.

Gene therapy • Treatment of a genetic disease by providing patients with cells containing wild type alleles for the genes that are nonfunctional in their bodies.

Generative nucleus • In a pollen tube, a haploid nucleus that undergoes mitosis to produce the two sperm nuclei that participate in double fertilization. (Contrast with tube nucleus.)

Genet • The genetic individual of a plant that is composed of a number of nearly identical but repeated units.

Genetic drift • Changes in gene frequencies from generation to generation in a small population as a result of random processes.

Genetic stochasticity • Variation in the frequencies of alleles and genotypes in a population over time.

Genetics • The study of heredity.

Genetic structure • The frequencies of alleles and genotypes in a population.

Genome (jee' nome) • The genes in a complete haploid set of chromosomes.

Genotype (jean' oh type) [Gr. gen: to produce + typos: impression] • An exact description of the genetic constitution of an individual, either with respect to a single trait or with respect to a larger set of traits. (Contrast with phenotype.)

Genus (jean' us) (plural: genera) [Gr. genos: stock, kind] • A group of related, similar species.

Geotropism • See gravitropism.

Germ cell • A reproductive cell or gamete of a multicellular organism.

Germination • The sprouting of a seed or spore.

Gestation (jes ray' shun) [L. gestare: to bear] • The period during which the embryo of a mammal develops within the uterus. Also known as pregnancy.

Gibberellin (jib er el' lin) [L. gibberella: hunchback (refers to shape of a reproductive structure of a fungus that produces gibberellins)] • One of a class of plant growth substances playing roles in stem elongation, seed germination, flowering of certain plants, etc. Named for the fungus *Gibberella*.

Gill • An organ for gas exchange in aquatic organisms.

Gill arch • A skeletal structure that supports gill filaments and the blood vessels that supply them.

Gizzard (giz' erd) [L. gigeria: cooked chicken parts] • A very muscular part of the stomach of birds that grinds up food, sometimes with the aid of fragments of stone.

Gland • An organ or group of cells that produces and secretes one or more substances.

Glans penis • Sexually sensitive tissue at the tip of the penis.

Glia (glee' uh) [Gr.: glue] • Cells, found only in the nervous system, which do not conduct action potentials.

Glomerulus (glo mare' yew lus) [L. glomus: ball] • Sites in the kidney where blood filtration takes place. Each glomerulus consists of a knot of capillaries served by afferent and efferent arterioles.

Glucocorticoids • Steroid hormones produced by the adrenal cortex. Secreted in response to ACTH, they inhibit glucose uptake by many tissues in addition to mediating other stress responses.

Glucagon • A hormone produced and released by cells in the islets of Langerhans of the pancreas. It stimulates the breakdown of glycogen in liver cells.

Gluconeogenesis • The biochemical synthesis of glucose from other substances, such as amino acids, lactate, and glycerol.

Glucose (glue' kose) [Gr. gleukos: sweet wine mash for fermentation] • The most common sugar, one of several monosaccharides with the formula $C_6H_{12}O_6$.

Glycerol (gliss' er ole) • A three-carbon alcohol with three hydroxyl groups, the linking component of phospholipids and triglycerides.

Glycogen (gly' ko jen) • A branched-chain polymer of glucose, similar to starch (which is less branched and may be of lower molecular weight). Exists mostly in liver and muscle; the principal storage carbohydrate of most animals and fungi.

Glycolysis (gly kol' li sis) [from glucose + Gr. lysis: loosening] • The enzymatic breakdown of glucose to pyruvic acid. One of the oldest energy-yielding mechanisms in living organisms.

Glycosidic linkage • The connection in an oligosaccharide or polysaccharide chain, formed by removal of water during the linking of monosaccharides by root pressure.

Glyoxysome (gly ox' ee soam) • An organelle found in plants, in which stored lipids are converted to carbohydrates.

Golgi apparatus (goal' jee) • A system of concentrically folded membranes found in the cytoplasm of eukaryotic cells. Plays a role in the production and release of secretory materials such as the digestive enzymes manufactured in the pancreas. First described by Camillo Golgi (1844-1926).

Gonad (go' nad) [Gr. gone: seed, that which produces seed] • An organ that produces sex cells in animals: either an ovary (female gonad) or testis (male gonad).

Gonadotropin • A hormone that stimulates the gonads.

Gondwana • The large southern land mass that existed from the Cambrian (540 mya) to the Jurassic (138 mya). Present-day South America, Africa, India, Australia, and Antarctica.

Gram stain • A differential stain useful in characterizing bacteria.

Granum • Within a chloroplast, a stack of thylakoids.

Gravitropism • A directed plant growth response to gravity.

Grazer • An animal that eats the vegetative tissues of herbaceous plants.

Green gland • An excretory organ of crustaceans.

Greenhouse effect • The heating of Earth's atmosphere by gases that are transparent to sunlight but opaque to radiated heat.

Gross primary production • The total energy captured by plants growing in a particular area.

Ground meristem • That part of an apical meristem that gives rise to the ground tissue system of the primary plant body

Ground tissue system • Those parts of the plant body not included in the dermal or vascular tissue systems. Ground tissues function in storage, photosynthesis, and support.

GLOSSARY

Group transfer • The exchange of atoms between molecules.

Growth • Irreversible increase in volume (probably the most accurate definition, but at best a dangerous oversimplification).

Growth factors • A group of proteins that circulate in the blood and trigger the normal growth of cells. Each growth factor acts only on certain target cells.

Guanine (gwan'een) • A nitrogen-containing base found in DNA, RNA and GTP.

Guard cells • In plants, paired epidermal cells which surround and control the opening of a stoma (pore).

Gut • An animal's digestive tract.

Guttation • The extrusion of liquid water through openings in leaves, caused by root pressure.

Gymnosperm (jim' no sperm) [Gr. gymnos: naked + sperma: seed] • A plant, such as a pine or other conifer, whose seeds do not develop within an ovary (hence, the seeds are "naked").

Gyrus (plural: gyri) • The raised or ridged portion of the convoluted surface of the brain. (Contrast to sulcus.)

Habit • The form or pattern of growth characteristic of an organism.

Habitat • The environment in which an organism lives.

Habituation (ha bich' oo ay shun) • The simplest form of learning, in which an animal presented with a stimulus without reward or punishment eventually ceases to respond.

Hair cell • A type of mechanoreceptor in animals.

Half-life • The time required for half of a sample of a radioactive isotope to decay to its stable, nonradioactive form.

Halophyte (hal' oh fyte) [Gr. Jmlos: salt + pin/ton: plant] • A plant that grows in a saline (salty) environment.

Haploid (hap' loid) [Gr. haploides: single] • Having a chromosome complement consisting of just one copy of each chromosome. This is the normal "ploidy" of gametes or of asexual spores produced by meiosis or of organisms (such as the

gametophyte generation of plants) that grow from such spores without fertilization.

Hardy-Weinberg equilibrium • The percentages of diploid combinations expected from a knowledge of the proportions of alleles in the population if no agents of evolution are acting on the population.

Haustorium (haw stor' ee um) [L. haustus: draw up] • A specialized hypha or other structure by which fungi and some parasitic plants draw food from a host plant.

Haversian systems • Units of organization in compact bone that reflect the action of intercommunicating osteoblasts.

Heat-shock proteins • Chaperone proteins expressed in cells exposed to high temperatures or other forms of environmental stress.

Helper T cells • T cells that participate in the activation of B cells and of other T cells; targets of the HIV-I virus, the agent of AIDS. (Contrast with cytotoxic T cells, suppressor T cells.)

Hematocrit (heme at o krit) [Gr. haima: blood + krites: judge] • The proportion of 100 cc of blood that consists of red blood cells.

Hemizygous (hem' ee zie' gus) [Gr. hemi: half + zygos: joined] • In a diploid organism, having only one allele for a given trait, typically the case for X-linked genes in male mammals and Z-linked genes in female birds. (Contrast with homozygous, heterozygous.)

Hemoglobin (hee' mo glow' bin) [Gr. haima: blood + L. globus: globe] • The colored protein of vertebrate blood (and blood of some invertebrates) which transports oxygen.

Hepatic (heh pat' ik) [Gr. liepar: liver] • Pertaining to the liver.

Hepatic duct • The duct that conveys bile from the liver to the gallbladder.

Herbicide (ur' bis ide) • A chemical substance that kills plants.

Herbivore [L. herba: plant + vorare: to devour] • An animal which eats the tissues of plants. (Contrast with carnivore, detritivore, omnivore.)

Heritable • Able to be inherited; in biology usually refers to genetically determined traits.

Hermaphroditism (her maf row dite' ism) [Gr. Iiermaphroditos: a person with both male and female traits] • The coexistence of both female and male sex organs in the same organism.

Hertz (abbreviated as Hz) • Cycles per second.

Hetero- [Gr.: other, different] • A prefix used in biology to mean that two or more different conditions are involved; for example, heterotroph, heterozygous.

Heterochromatin • Chromatin that retains its coiling during interphase; generally not transcribed. (Contrast with euchromatin.)

Heterocyst • A large, thick-walled cell in the filaments of certain cyanobacteria; performs nitrogen fixation.

Heterogeneous nuclear RNA (hnRNA) •

The product of transcription of a eukaryotic gene, including transcripts of introns.

Heteromorphic (hef er oh more' fik) [Gr. heteros: different + morphe: form] • having a different form or appearance, as two heteromorphic life stages of a plant. (Contrast with isomorphic.)

Heterosporous (het' er os' por us) • Producing two types of spores, one of which gives rise to a female megaspore and the other to a male microspore. Heterosporous plants produce distinct female and male gametophytes. (Contrast with homosporous.)

Heterotherm • An animal that regulates its body temperature at a constant level at some times but not others, such as a hibernator.

Heterotroph (het' er oh trof) [Gr. heteros: different + trophic: food] • An organism that requires preformed organic molecules as food. (Contrast with autotroph.)

Heterozygous (het' er oh zie' gus) [Gr. heteros: different + zygos: joined] • Of a diploid organism having different alleles of a given gene on the pair of homologues carrying that gene. (Contrast with homozygous.)

Hibernation [L. hibernans: winter] • The state of inactivity of some animals during winter; marked by a drop in body temperature and metabolic rate.

Highly repetitive DNA • Short DNA sequences present in millions of copies in the genome, next to each other (in tandem). In a reassociation experiment, denatured highly repetitive DNA reanneals very quickly.

Hippocampus • A part of the forebrain that takes part in long-term memory formation.

Histamine (hiss; tah meen) • A substance released within a damaged tissue by a type of white blood cell. Histamines are responsible for aspects of allergic reactions, including the increased vascular permeability that leads to edema (swelling).

Histology • The study of tissues.

Histone • Any one of a group of basic proteins forming the core of a nucleosome, the structural unit of a eukaryotic chromosome. (See nucleosome.)

hnRNA • See heterogeneous nuclear RNA.

Homeobox • A 180-base-pair segment of DNA found in a few genes (called Hox genes), perhaps regulating the expression of other genes and thus controlling large-scale developmental processes.

Homeostasis (home' ee o sta' sis) [Gr. homos: same + stasis: position] • The maintenance of a steady state, such as a constant temperature or a stable social structure, by means of physiological or behavioral feedback responses.

Homeotherm (home' ee o therm) [Gr. homos: same + therme: heat] • An animal which maintains a constant body temperature by virtue of its own heating and cooling mechanisms. (Contrast with heterotherm, poik-ilotherm.)

Homeotic genes (home' ee ott' ic) • Genes that determine what entire segments of an animal become. Drastic mutations in these genes cause the transformation of body segments in *Drosophila*. Homeotic genes studied in the plant *Arabidopsis* are called organ identity genes.

Homolog (home' o log') [Gr. homos: same + logos: word] • One of a pair, or larger set, of chromosomes having the same overall genetic composition and sequence. In diploid organisms, each chromosome inherited from one parent is matched by an identical (except for mutational changes) chromosome—its homolog—from the other parent.

Homology (ho mol' o jee) [Gr. homologí(a): agreement] • A similarity between two structures that is due to inheritance from a common ancestor. The structures are said to be homologous (Contrast with analogy.)

Homoplasmy (home' uh plaj zee) [Gr. homos: same + plastikos; to mold] • The presence in several species of a trait not present in their most common ancestor. Can result from convergent evolution, reversion, or parallel evolution

Homozygous • Producing a single type of spore that gives rise to a single type of gametophyte, bearing both female and male reproductive organs. (Contrast with heterozygous.)

Homozygous (home' o zie' gus) [Gr. homos: same + zygotes: joined] • Of a diploid

organism having identical alleles of a given gene on both homologous chromosomes. An organism may be a "homozygote" with respect to one gene and, at the same time, a "heterozygote" with respect to another. (Contrast with heterozygous.)

Hormone (hore' mone) [Gr. hormon: excite, stimulate] • A substance produced in one part of a multicellular organism and transported to another part where it exerts its specific effect on the physiology or biochemistry of the target cells.

Host • An organism that harbors a parasite and provides it with nourishment.

Host-parasite interaction • The dynamic interaction between populations of a host and the parasites that attack it.

Hox genes • See homeobox.

Humoral immune system • The part of the immune system mediated by B cells; it is mediated by circulating antibodies and is active against extracellular bacterial and viral infections.

Humus (hew' muss) • The partly decomposed remains of plants and animals on the surface of a soil. Its characteristics depend primarily upon climate and the species of plants growing on the site.

Hyaluronidase (hill yew ron' uh dase) • An enzyme that digests proteoglycans. Found in sperm cells, it helps digest the coatings surrounding an egg so the sperm can penetrate the egg cell membrane.

Hybrid (high' brid) [L. hybrida: mongrel] • The offspring of genetically dissimilar parents. In molecular biology, a double helix formed of nucleic acids from different sources.

Hybridoma • A cell produced by the fusion of an antibody-producing cell with a myeloma cell; it produces monoclonal antibodies.

Hybrid zone • A narrow zone where two populations interbreed, producing hybrid individuals.

Hydrocarbon • A compound containing only carbon and hydrogen atoms.

Hydrogen bond • A chemical bond which arises from the attraction between the slight positive charge on a hydrogen atom

and a slight negative charge on a nearby fluorine, oxygen, or nitrogen atom. Weak bonds, but found in great quantities in proteins, nucleic acids, and other biological macromolecules.

Hydrological cycle • The sum total of movement of water from the oceans to the atmosphere, to the soil, and back to the oceans. Some water is cycled many times within compartments of the system before completing one full circuit.

Hydrolyze (hi' dro lize) [Gr. hydro: water + lysis: cleavage] • To break a chemical bond, as in a peptide linkage, with the insertion of the components of water, -H and -OH, at the cleaved ends of a chain. The digestion of proteins is a hydrolysis.

Hydrophilic [Gr. hydro: water + philia: love] • Having an affinity for water. (Contrast with hydrophobic.)

Hydrophobic [Gr. hydro: water + phobia: fear] • Molecules and amino acid side chains, which are mainly hydrocarbons (compounds of C and H with no charged groups or polar groups), have a lower energy when they are clustered together than when they are distributed through an aqueous solution. Because of their attraction for one another and their reluctance to mix with water they are called "hydrophobic." Oil is a hydrophobic substance; phenylalanine is a hydrophobic amino acid in a protein. (Contrast with hydrophilic.)

Hydrostatic skeleton • The incompressible internal liquids of some animals that transfer forces from one part of the body to another when acted upon by the surrounding muscles.

Hydroxyl group • The —OH group, characteristic of alcohols.

Hyperpolarization • A change in the resting potential of a membrane so the inside of a cell becomes more electronegative. (Contrast with depolarization.)

Hypersensitive response • A defensive response of plants to microbial infection; it results in a "dead spot."

Hypertension • High blood pressure.

Hypertonic [Gk. hyper: above, over] • Having a greater solute concentration. Said of one solution in comparing it to another. (Contrast with hypotonic, isotonic.)

Hypha (high' fuh) (plural: hyphae) [Gr. hyphe: web] • In the fungi, any single filament. May be multinucleate (zygomycetes, ascomycetes) or multicellular (basid-iomycetes).

Hypocotyl [Gk. hypo: beneath, under + kotyledon: hollow space] • That part of the embryonic or seedling plant shoot that is below the cotyledons.

Hypothalamus • The part of the brain lying below the thalamus; it coordinates water balance, reproduction, temperature regulation, and metabolism.

Hypothesis • A tentative answer to a question, from which testable predictions can be generated. (Contrast with theory.)

Hypothetico-deductive method • A

method of science in which hypotheses are erected, predictions are made from them, and experiments and observations are performed to test the predictions.

Hypotonic [Gk. hypo: beneath, under] • Having a greater solute concentration. Said of one solution in comparing it to another. (Contrast with hypotonic, isotonic.)

Imaginal disc • In insect larvae, groups of cells that develop into specific adult organs.

Immune system [L. immunis: exempt] • A system in mammals that recognizes and eliminates or neutralizes either foreign substances or self substances that have been altered to appear foreign.

Immunization • The deliberate introduction of antigen to bring about an immune response.

Immunoglobulins • A class of proteins, with a characteristic structure, active as receptors and effectors in the immune system.

Immunological memory • Certain clones of immune system cells made to respond to an antigen persist. This leads to a more rapid and massive response of the immune system to any subsequent exposure to that antigen.

Immunological tolerance • A mechanism by which an animal does not mount an immune response to the antigenic determinants of its own macromolecules.

Imprinting • (1) In genetics, the differential modification of a gene depending on whether it is present in a male or a female. (2) In animal behavior, a rapid form of learning in which an animal comes to make a particular response, which is maintained for life, to some object or other organism.

Inclusive fitness • The sum of an individual's own fitness (the effect of producing its own offspring: the individual selection component) plus its influence on fitness in relatives other than direct descendants (the kin selection component).

Incomplete dominance • Condition in which the heterozygous phenotype is intermediate between the two homozygous phenotypes.

Incomplete metamorphosis • Insect development in which changes between instars are gradual.

Incus (in' kus) [L. incus: anvil] • The middle of the three bones that conduct movements of the eardrum to the oval window of the inner ear. (See malleus, stapes.)

Independent assortment • The random separation during meiosis of nonhomologous chromosomes and of genes carried on nonhomologous chromosomes.

Individual fitness • That component of inclusive fitness that results from an organism producing its own offspring. (Contrast with kin selection component.)

Indoleacetic acid • See auxin.

Inducer • (1) In enzyme systems, a small molecule which, when added to a growth medium, causes a large increase in the level of some enzyme. (2) In embryology, a substance that causes a group of target cells to differentiate in a particular way.

Inducible enzyme • An enzyme that is present in much larger amounts when a particular compound (the inducer) has been

GLOSSARY

added to the system. (Contrast with constitutive enzyme.)

Inflammation • A nonspecific defense against pathogens; characterized by redness, swelling, pain, and increased temperature.

Inflorescence • A structure composed of several flowers.

Inhibitor • A substance which binds to the surface of an enzyme and interferes with its action on its substrates.

Inhibitory postsynaptic potential • A

change in the resting potential of a postsynaptic membrane in the hyperpolarizing (negative) direction.

Initiation complex • Combination of a ribosomal light subunit, an mRNA molecule, and the tRNA charged with the first amino acid coded for by the mRNA; formed at the onset of translation.

Initiation factors • Proteins that assist in forming the translation initiation complex at the ribosome.

Inositol triphosphate (IP₃) • An intracellular second messenger derived from membrane phospholipids.

Instar (in' star) [L.: image, form] • An immature stage of an insect between molts.

Insulin (in' su lin) [L. insula: island] • A hormone, synthesized in islet cells of the pancreas, that promotes the conversion of glucose to the storage material, glycogen.

Integrase • An enzyme that integrates retroviral cDNA into the genome of the host cell.

Integrated pest management • A method of control of pests in which natural predators and parasites are used in conjunction with sparing use of chemical methods to achieve control of a pest without causing serious adverse environmental side effects.

Integument [L. integumentum: covering] • A protective surface structure. In gymnosperms and angiosperms, a layer of tissue around the ovule which will become the seed coat. Gymnosperm ovules have one integument, angiosperm ovules two.

Intercalary meristem • A meristematic region in plants which occurs not apically, but between two regions of mature tissue. Intercalary meristems occur in the nodes of grass stems, for example.

Intercostal muscles • Muscles between the ribs that can augment breathing movements by elevating and suppressing the rib cage.

Interferon • A glycoprotein produced by virus-infected animal cells; increases the resistance of neighboring cells to the virus.

Interkinesis • The phase between the first and second meiotic divisions.

Interleukins • Regulatory proteins, produced by macrophages and lymphocytes, that act upon other lymphocytes and direct their development.

Intermediate filaments • Fibrous proteins that stabilize cell structure and resist tension.

Internode • Section between two nodes of a plant stem.

Interphase • The period between successive nuclear divisions during which the chromosomes are diffuse and the nuclear envelope is intact. It is during this period that the cell is most active in transcribing and translating genetic information.

Interspecific competition • Competition between members of two or more species.

Intertropical convergence zone • The tropical region where the air rises most strongly; moves north and south with the passage of the sun overhead.

Intraspecific competition • Competition among members of a single species.

Intrinsic protein • A membrane protein that is embedded in the phospholipid bilayer of the membrane. (Contrast with extrinsic protein.)

Intrinsic rate of increase • The rate at which a population can grow when its density is low and environmental conditions are highly favorable.

Intron • A portion of a DNA molecule that, because of RNA splicing, is not involved in coding for part of a polypeptide molecule. (Contrast with exon.)

Invagination • An infolding.

Inversion (genetic) • A rare mutational event that leads to the reversal of the order of genes within a segment of a chromosome, as if that segment had been removed from the chromosome, turned 180°, and then reattached.

Invertebrate • Any animal that is not a vertebrate, that is, whose nerve cord is not enclosed in a backbone of bony segments.

In vitro [L.: in glass] • In a test tube, rather than in a living organism. (Contrast with *in vivo*.)

In vivo [L.: in the living state] • In a living organism. Many processes that occur *in vivo* can be reproduced *in vitro* with the right selection of cellular components. (Contrast with *in vitro*.)

Ion (eye' on) [Gr.: wanderer] • An atom or group of atoms with electrons added or removed, giving it a negative or positive electrical charge.

Ion channel • A membrane protein that can let ions pass across the membrane. The channel can be ion-selective, and it can be voltage-gated or ligand-gated.

Ionic bond • A chemical bond which arises from the electrostatic attraction between positively and negatively charged ions. Usually a strong bond.

Iris (eye' ris) [Gr. iris: rainbow] • The round, pigmented membrane that surrounds the pupil of the eye and adjusts its aperture to regulate the amount of light entering the eye.

Irruption • A rapid increase in the density of a population. Often followed by massive emigration.

Islets of Langerhans • Clusters of hormone-producing cells in the pancreas.

Iso- [Gr.: equal] • Prefix used to denote two separate but similar or identical states of a characteristic. (See isomers, isomorphic, isotope.)

Isolating mechanism • Geographical, physiological, ecological, or behavioral mechanisms that lead to a reduction in the frequency of hybrid matings.

Isomers • Molecules consisting of the same numbers and kinds of atoms, but differing in the way in which the atoms are combined.

Isomorphic (eye' so more' fik) [Gr. isos: equal + morphe: form] • having the same form or appearance, as two isomorphic life stages. (Contrast with heteromorphic.)

Isotonic • Having the same solute concentration; said of two solutions. (Contrast with hypertonic, hypotonic.)

Isotope (eye' so tope) [Gr. isos: equal + topos: place] • Two isotopes of the same chemical element have the same number of protons in their nuclei, but differ in the number of neutrons.

Jasmonates • Plant hormones that trigger defenses against pathogens and herbivores.

Jejunum (jih jew' num) • The middle division of the small intestine, where most absorption of nutrients occurs. (See duodenum, ileum.)

Joule (jool, or jowl) • A unit of energy, equal to 0.24 calories.

Juvenile hormone • In insects, a hormone maintaining larval growth and preventing maturation or pupation.

Karyotype • The number, forms, and types of chromosomes in a cell.

Kelvin temperature scale • See absolute temperature scale.

Keratin (ker' a tin) [Gr. keras: horn] • A protein which contains sulfur and is part of such hard tissues as horn, nail, and the outermost cells of the skin.

Ketone (key' tone) • A compound with a C=O group attached to two other groups, neither of which is an H atom. Many sugars are ketones. (Contrast with aldehyde.)

Keystone species • A species that exerts a major influence on the composition and dynamics of the community in which it lives.

Kidneys • A pair of excretory organs in vertebrates.

Kin selection • The component of inclusive fitness resulting from helping the survival of relatives containing the same alleles by descent from a common ancestor.

Kinase (kye' nase) • An enzyme that transfers a phosphate group from ATP to another molecule. Protein kinases transfer phosphate from ATP to specific proteins, playing important roles in cell regulation.

Kinesis (ki nee' sis) [Gr.: movement] • Orientation behavior in which the organism does not move in a particular direction with reference to a stimulus but instead simply moves at an increasing or decreasing rate until it ends up farther from the object or closer to it. (Contrast with taxis.)

SSAR>

Kinetochore (km not' oh core) [Gr kinetos: moving + khorein: to move] • Specialized structure on a centromere to which microtubules attach.

Koch's postulates • Four rules For establishing that a particular microorganism causes a particular disease.

Krebs cycle • See citric acid cycle.

Lactic acid • The end product of fermentation in vertebrate muscle and some microorganisms.

Lagging strand • In DNA replication, the daughter strand that is synthesized discontinuously.

Lamella • Layer.

Larynx (lar' inks) • A structure between the pharynx and the trachea that includes the vocal cords.

Larva (plural: larvae) [L.: ghost, early stage] • An immature stage of any invertebrate animal that differs dramatically in appearance from the adult.

Lateral • Pertaining to the side.

Lateral gene transfer • The movement of genes from one prokaryotic species to another.

Lateral meristems • The vascular cambium and cork cambium, which give rise to secondary tissue in plants.

Laterization (lat' ur iz ay shun) • The formation of a nutrient-poor soil that is rich in insoluble iron and aluminum compounds.

Law of independent assortment • The random separation during meiosis of nonhomologous chromosomes and of genes carried on nonhomologous chromosomes. Mendel's second law.

Law of segregation • Alleles segregate from one another during gamete formation, Mendel's first law.

Leader sequence • A sequence of amino acids at the N-terminal end of a newly synthesized protein, determining where the protein will be placed in the cell.

Leading strand • In DNA replication, the daughter strand that is synthesized continuously.

Lenticel • Spongy region in a plant's periderm, allowing gas exchange.

Leukocyte (loo' ko sight) [Gr. leukos: clear + kutos: hollow vessel] • A white blood cell.

Lichen (lie' kun) [Gr. leikhn: lick] • An organism resulting from the symbiotic association of a true fungus and either a cyanobacterium or a unicellular alga.

Life cycle • The entire span of the life of an organism from the moment of fertilization (or asexual generation) to the time it reproduces in turn.

Life history • The stages an individual goes through during its life.

Life table • A table showing, for a group of equal-aged individuals, the proportion still alive at different times in the future and the number of offspring they produce during each time interval.

Ligament • A band of connective tissue linking two bones in a joint.

Ligand (lig' and) • A molecule that binds to a receptor site of another molecule.

Lignin • The principal noncarbohydrate component of wood, a polymer that binds together cellulose fibrils in some plant cell

walls.

Limbic system • A group of primitive vertebrate forebrain nuclei that form a network and are involved in emotions, drives, instinctive behaviors, learning, and memory-Limiting resource • The required resource whose supply most strongly influences the size of a population.

Linkage • Association between genetic markers on the same chromosome such that they do not show random assortment and seldom recombine; the closer the markers, the lower the frequency of recombination.

Lipase (lip' ase; lye' pase) • An enzyme that digests fats.

Lipids (lip' ids) [Gr. lipos: fat] • Substances in a cell which are easily extracted by organic solvents; fats, oils, waxes, steroids, and other large organic molecules, including those which, with proteins, make up the cell membranes. (See phospholipids.)

Litter • The partly decomposed remains of plants on the surface and in the upper layers of the soil.

Littoral zone • The coastal zone from the upper limits of tidal action down to the depths where the water is thoroughly stirred by wave action.

Liver • A large digestive gland. In vertebrates, it secretes bile and is involved in the formation of blood.

Lobes • Regions of the human cerebral hemispheres; includes the temporal, frontal, parietal, and occipital lobes.

Locus • In genetics, a specific location on a chromosome. May be considered to be synonymous with "gene."

Logistic growth • Growth, especially in the size of an organism or in the number of organisms that constitute a population, which slows steadily as the entity approaches its maximum size. (Contrast with exponential growth.)

Loop of Henle (hen' lee) • Long, hairpin loop of the mammalian renal tubule that runs from the cortex down into the medulla, and back to the cortex. Creates a concentration gradient in the interstitial fluids in the medulla.

Lophophore • A U-shaped fold of the body wall with hollow, ciliated tentacles that encircles the mouth of animals in several different phyla. Used for filtering prey from the surrounding water.

Lordosis (lor doe' sis) [Gk. lordosis: curving forward] • A posture assumed by females of some mammalian species (especially rodents) to signal sexual receptivity.

Lumen (loo' men) [L.: light] • The cavity inside any tubular part of an organ, such as a piece of gut or a kidney tubule.

Lungs • A pair of saclike chambers within the bodies of some animals, functioning in gas exchange.

Luteinizing hormone • A gonadotropin produced by the anterior pituitary. It stimulates the gonads to produce sex hormones.

Lymph [L. lymphā: water] • A clear, watery fluid that is formed as a filtrate of blood; it contains white blood cells; it collects in a series of special vessels and is returned to the bloodstream.

Lymph nodes • Specialized tissue regions that act as filters for cells, bacteria and foreign matter.

Lymphocyte • A major class of white blood cells. Includes T cells, B cells, and other cell types important in the immune response.

Lysis (lie' sis) [Gr.: a loosening] • Bursting of a cell.

Lysogenic • The condition of a bacterium that carries the genome of a virus in a relatively stable form. (Contrast with lytic.)

Lysosome (lie' so soam) [Gr. lysis: a loosening + soma: body] • A membrane-bounded inclusion found in eukaryotic cells (other than plants). Lysosomes contain a mixture of enzymes that can digest most of the macromolecules found in the rest of the cell.

Lysozyme (lie' so zyme) • An enzyme in saliva, tears, and nasal secretions that attacks bacterial cell walls, as one of the body's nonspecific defense mechanisms.

Lytic • Condition in which a bacterium lyses shortly after infection by a virus; the viral genome does not become stabilized within the bacterial cell. (Contrast with lysogenic.)

Macro- (mack' roh) [Gr. makros: large, long]

• A prefix commonly used to denote something large. (Contrast with micro-.)

Macroevolution • Evolutionary changes occurring over long time spans and usually involving changes in many traits. (Contrast with microevolution.)

Macromolecule • A giant polymeric molecule. The macromolecules are proteins, polysaccharides, and nucleic acids.

Macronutrient • A mineral element required by plant tissues in concentrations of at least 1 milligram per gram of their dry

matter.

Macrophage (mac' roh faj) • A type of white blood cell that endocytoses bacteria and other cells.

Major histocompatibility complex (MHC)

• A complex of linked genes, with multiple alleles, that control a number of immunological phenomena; it is important in graft rejection.

Malignant tumor • A tumor whose cells can invade surrounding tissues and spread to other organs.

Malleus (mal' ee us) [L. malleus: hammer] • The first of the three bones that conduct movements of the eardrum to the oval window of the inner ear. (See incus, stapes.)

Malpighian tubule (mal pee' gy un) • A type of protonephridium found in insects.

GLOSSARY

Mammal [L. mamma: breast, teat] • Any animal of the class Mammalia, characterized by the production of milk by the female mammary glands and the possession of hair for body covering.

Mantle • A sheet of specialized tissues that covers most of the viscera of mollusks; provides protection to internal organs and secretes the shell.

Map unit • In eukaryotic genetics, one map unit corresponds to a recombinant frequency of 0.01.

Mapping • In genetics, determining the order of genes on a chromosome and the distances between them.

Marine [L. mare: sea, ocean] • Pertaining to or living in the ocean. (Contrast with aquatic, terrestrial.)

Marsupial (mar soo' pee al) • A mammal belonging to the subclass Metatheria, such as opossums and kangaroos. Most have a pouch (marsupium) that contains the milk glands and serves as a receptacle for the young.

Mass extinctions • Geological periods during which rates of extinction were much higher than during intervening times.

Mass number • The sum of the number of protons and neutrons in an atom's nucleus.

Mast cells • Typically found in connective tissue, mast cells can be provoked by antigens or inflammation to release histamine.

Maternal effect genes • These genes code for morphogens that determine the polarity of the egg and larva in the fruit fly, *Drosophila melanogaster*.

Maternal inheritance (cytoplasmic inheritance) • Inheritance in which the phenotype of the offspring depends on factors, such as mitochondria or chloroplasts, that are inherited from the female parent through the cytoplasm of the female gamete.

Maturation • The automatic development of a pattern of behavior, which becomes increasingly complex or precise as the animal matures. Unlike learning, the development does not require experience to occur.

Mechanoreceptor • A cell that is sensitive to physical movement and generates action potentials in response.

Medulla (meh dull' luh) [L.: narrow] • (1) The inner, core region of an organ, as in the adrenal medulla (adrenal gland) or the renal medulla (kidneys). (2) The portion of the brain stem that connects to the spinal cord.

Mega- [Gr. megas: large, great] • A prefix often used to denote something large. (Contrast with micro-.)

Megaspore [Gr. megas: large + sporaseed] • In plants, a haploid spore that produces a female gametophyte.

Meiosis (my oh' sis) [Gr.: diminution] • Division of a diploid nucleus to produce four haploid daughter cells. The process consists of two successive nuclear divisions with only one cycle of chromosome replication.

Membrane potential • The difference in electrical charge between the inside and the outside of a cell, caused by a difference in the distribution of ions.

Memory cells • Long-lived lymphocytes produced by exposure to antigen. They persist in the body and are able to mount a rapid response to subsequent exposures to the antigen.

Mendelian population • A local population of individuals belonging to the same species and exchanging genes with one another.

Menopause • The time in a human female's life when the ovarian and menstrual cycles cease.

Menstrual cycle • The monthly sloughing off of the uterine lining if fertilization does not occur in the female. Occurs between puberty and menopause.

Meristem [Gr. meristos: divided] • Plant tissue made up of actively dividing cells.

Mesenchyme (mez' en kyme) [Gr. mesos: middle + enchyma: infusion] • Embryonic or unspecialized cells derived from the mesoderm.

Meso- (mez' oh) [Gr.: middle] • A prefix often used to designate a structure located in the middle, or a stage that appears at some intermediate time. For example, mesoderm, Mesozoic.

Mesoderm [Gr. mesos: middle + derma: skin] • The middle of the three embryonic tissue layers first delineated during gastrulation. Gives rise to skeleton, circulatory system, muscles, excretory system, and most of the reproductive system.

Mesophyll (mez' a fill) [Gr. mesos: middle + phyllon: leaf] • Chloroplast-containing, photo-synthetic cells in the interior of leaves.

Mesosome (mez' o soam') [Gr. mesos: middle + soma: body] • A localized infolding of the plasma membrane of a bacterium.

Messenger RNA (mRNA) • A transcript of one of the strands of DNA, it carries information (as a sequence of codons) for the synthesis of one or more proteins.

Meta- [Gr.: between, along with, beyond] • A prefix used in biology to denote a change or a shift to a new form or level; for example, as used in metamorphosis.

Metabolic compensation • Changes in biochemical properties of an organism that render it less sensitive to temperature changes.

Metabolic pathway • A series of enzyme-catalyzed reactions so arranged that the product of one reaction is the substrate of the next.

Metabolism (meh tab' a lizm) [Gr. metabole: to change] • The sum total of the chemical reactions that occur in an organism, or some subset of that total (as in "respiratory metabolism").

Metamorphosis (met' a mor' fo sis) [Gr. meta: between + morphe: form, shape] • A radical change occurring between one developmental stage and another, as for example from a tadpole to a frog or an insect larva to the adult.

Metaphase (met' a phase) [Gr. meta: between] • The stage in nuclear division at which the centromeres of the highly super-coiled chromosomes are all lying on a plane

(the metaphase plane or plate) perpendicular to a line connecting the division poles.

Metapopulation • A population divided into subpopulations, among which there are occasional exchanges of individuals.

Metastasis (meh tass' tuh sis) • The spread of cancer cells from their original site to other parts of the body.

Methanogen • Any member of a group of Archaeobacteria that release methane as a metabolic product. This group is considered to be an extremely ancient one.

MHC • See major histocompatibility complex.

Micro- (mike' roh) [Gr. mikros: small] • A prefix often used to denote something small. (Contrast with macro-, mega-.)

Microbiology [Gr. mikros: small + bios: life + logos: discourse] • The scientific study of microscopic organisms, particularly bacteria, unicellular algae, protists, and viruses.

Microevolution • The small evolutionary changes typically occurring over short time spans; generally involving a small number of traits and minor genetic changes. (Contrast with macroevolution.)

Microfilament • Minute fibrous structure generally composed of actin found in the cytoplasm of eukaryotic cells. They play a role in the motion of cells.

Micronutrient • A mineral element required by plant tissues in concentrations of less than 100 micrograms per gram of their dry matter.

Micropyle (mike' roh pile) [Gr. mikros: small + pyle: gate] • Opening in the integument(s) of a seed plant ovule through which pollen grows to reach the female gametophyte within.

Microspores [Gr. mikros: small + spora: seed] • In plants, a haploid spore that produces a male gametophyte.

Microtubules • Minute tubular structures found in centrioles, spindle apparatus, cilia, flagella, and other places in the cytoplasm of eukaryotic cells. These tubules play roles in the motion and maintenance of shape of eukaryotic cells.

Microvilli (singular: microvillus) • The projections of epithelial cells, such as the cells lining the small intestine, that increase their surface area.

Middle lamella • A layer of derivative polysaccharides that separates plant cells; a common middle lamella lies outside the primary walls of the two cells.

Migration • The regular, seasonal movements of animals between breeding and nonbreeding ranges.

Mimicry (mim' ik ree) • The resemblance of one kind of organism to another, or to some inanimate object; serves the function of making the organism difficult to find, of discouraging potential enemies or of attracting potential prey (See Batesian mimicry and Mullerian mimicry.)

Mineral • An inorganic substance other than water.

GLOSSAR\

Mineralocorticoid • A hormone produced

by the adrenal cortex that influences mineral ion balance; aldosterone.

Mismatch repair • When a single base in DNA is changed into a different base, or the wrong base inserted during DNA replication, there is a mismatch in base pairing with the base on the opposite strand. A repair system removes the incorrect base and inserts the proper one for pairing with the opposite strand.

Missense mutation • A nonsynonymous mutation, or one that changes a codon for one amino acid to a codon for a different amino acid. (Contrast with frame-shift mutation, nonsense mutation, synonymous mutation.)

Mitochondrial matrix • The fluid interior of the mitochondrion, enclosed by the inner mitochondrial membrane.

Mitochondrion (my' toe kon' dree un) (plural: mitochondria) [Gr. mitos: thread + chondros: cartilage, or grain] • An organelle that occurs in eukaryotic cells and contains the enzymes of the citric acid cycle, the respiratory chain, and oxidative phosphorylation. A mitochondrion is bounded by a double membrane.

Mitosis (my toe' sis) [Gr. mitos: thread] • Nuclear division in eukaryotes leading to the formation of two daughter nuclei each with a chromosome complement identical to that of the original nucleus.

Mitotic center • Cellular region that organizes the microtubules for mitosis. In animals a centrosome serves as the mitotic center.

Moderately repetitive DNA • DNA

sequences that appear hundreds to thousands of times in the genome. They include the DNA sequences coding for rRNAs and tRNAs, as well as the DNA at telomeres.

Modular organism • An organism which grows by producing additional units of body construction (modules) that are very similar to the units of which it is already composed.

Mole • A quantity of a compound whose weight in grams is numerically equal to its molecular weight expressed in atomic mass units. Avogadro's number of molecules: 6.023×10^{23} molecules.

Molecular clock • The theory that macro-molecules diverge from one another over evolutionary time at a constant rate, and that discovering this rate gives insight into the phylogenetic relationships of organisms.

Molecular weight • The sum of the atomic weights of the atoms in a molecule.

Molecule • A particle made up of two or more atoms joined by covalent bonds or ionic attractions.

Molting • The process of shedding part or all of an outer covering, as the shedding of feathers by birds or of the entire exoskeleton by arthropods.

Mono- [Gr. *monos*: one] • Prefix denoting a single entity. (Contrast with *poly*.)

Monoclonal antibody • Antibody produced in the laboratory from a clone of hybridoma cells, each of which produces the same specific antibody.

Monocot (short for monocotyledon) [Gr. *monos*: one + *kotyledon*: a cup-shaped hollow] • Any member of the angiosperm class Monocotyledones, plants in which the embryo produces but a single cotyledon (seed leaf). Leaves of most monocots have their major veins arranged parallel to each other.

Monocytes • White blood cells that produce macrophages.

Monoecious (mo nee' shus) [Gr.: one house] • Organisms in which both sexes are "housed" in a single individual, which produces both eggs and sperm. (In some plants, these are found in different flowers within the same plant.) Examples: corn, peas, earthworms, hydras. (Contrast with dioecious, perfect flower.)

Monohybrid cross • A mating in which the parents differ with respect to the alleles of only one locus of interest.

Monomer [Gr.: one unit] • A small molecule, two or more of which can be combined to form oligomers (consisting of a few monomers) or polymers (consisting of many monomers).

Monophyletic (mon' oh fih leht' ik) [Gk. *monos*: single + *phylon*: tribe] • Being descended from a single ancestral stock.

Monosaccharide • A simple sugar. Oligosaccharides and polysaccharides are made up of monosaccharides.

Monosynaptic reflex • A neural reflex that begins in a sensory neuron and makes a single synapse before activating a motor neuron.

Morphogens • Diffusible substances whose concentration gradients determine patterns of development in animals and plants.

Morphogenesis (more' fo jen' e sis) [Gr. *morphe*: form + *genesis*: origin] • The development of form. Morphogenesis is the overall consequence of determination, differentiation, and growth.

Morphology (more fol' o jee) [Gr. *morphe*: form + *logos*: discourse] • The scientific study of organic form, including both its development and function.

Mosaic development • Pattern of animal embryonic development in which each blastomere contributes a specific part of the adult body. (Contrast with regulative development.)

Motor end plate • The modified area on a muscle cell membrane where a synapse is formed with a motor neuron.

Motor neuron • A neuron carrying information from the central nervous system to an effector such as a muscle fiber.

Motor unit • A motor neuron and the set of muscle fibers it controls.

mRNA • (See messenger RNA.)

Mucosa (mew koh' sah) • An epithelial membrane containing cells that secrete

mucus. The inner cell layers of the digestive and respiratory tracts.

Mullerian mimicry • The resemblance of two or more unpleasant or dangerous kinds of organisms to each other.

Multicellular [L. *multus*: much + *cella*: chamber] • Consisting of more than one cell, as for example a multicellular organism. (Contrast with unicellular.)

Muscle • Contractile tissue containing actin and myosin organized into polymeric chains called microfilaments. In vertebrates, the tissues are either cardiac muscle, smooth muscle, or striated (skeletal) muscle.

Muscle fiber • A single muscle cell. In the case of striated muscle, a syncytial, multinucleate cell.

Muscle spindle • Modified muscle fibers encased in a connective sheath and functioning as stretch receptors.

Mutagen (mute' ah jen) [L. mutare: change + Gr. genesis: source] • Any agent (e.g., chemicals, radiation) that increases the mutation rate.

Mutation • An inherited change along a very narrow portion of the nucleic acid sequence.

Mutation pressure • Evolution (change in gene proportions) by different mutation rates alone.

Mutualism • The type of symbiosis, such as that exhibited by fungi and algae or cyanobacteria in forming lichens, in which both species profit from the association.

Mycelium (my seel' ee yum) [Gr. mykes: fungus] • In the fungi, a mass of hyphae.

Mycorrhiza (my' ka rye' za) [Gr. mykes: fungus + rhiza: root] • An association of the root of a plant with the mycelium of a fungus.

Myelin (my' a lin) • A material forming a sheath around some axons. It is formed by Schwann cells that wrap themselves about the axon. It serves to insulate the axon electrically and to increase the rate of transmission of a nervous impulse.

Myofibril (my' oh fy' bril) [Gr. mys: muscle + L.fibrilla: small fiber] • A polymeric unit of actin or myosin in a muscle.

Myogenic (my oh jen' ik) [Gr. mys: muscle + genesis: source] • Originating in muscle.

Myoglobin (my' oh globe in) [Gr. mys: muscle + L. globus: sphere] • An oxygen-binding molecule found in muscle. Consists of a heme unit and a single globin chain, and carries less oxygen than hemoglobin.

Myosin [Gr. mys: muscle] • One of the two major proteins of muscle, it makes up the thick filaments. (See actin.)

NAD (nicotinamide adenine dinucleotide)

• A compound found in all living cells, existing in two interconvertible forms: the oxidizing agent NAD + and the reducing agent NADH.

NADP (nicotinamide adenine dinucleotide phosphate) • Like NAD, but possessing

.

GLOSSARY

another phosphate group; plays similar roles but is used by different enzymes.

Natural selection • The differential contribution of offspring to the next generation by various genetic types belonging to the same population. The mechanism of evolution proposed by Charles Darwin.

Necrosis (nee roh' sis) • Tissue damage resulting from cell death.

Negative control • The situation in which a regulatory macromolecule (generally a repressor) functions to turn off transcription. In the absence of a regulatory macro-molecule, the structural genes are turned on.

Nekton [Gr. neklein: to swim] • Animals, such as fish, that can swim against currents of water. (Contrast with plankton.)

Nematocyst (ne mat' o sist) [Gr. noun: thread + h/stis: cell] • An elaborate, threadlike structure produced by cells of jellyfish and other cnidarians, used chiefly to paralyze and capture prey.

Nephridium (nef rid' ee um) [Gr. nephros: kidney] • An organ which is involved in excretion, and often in water balance, involving a tube that opens to the exterior at one end.

Nephron (nef ron) [Gr. nephros: kidney] • The basic component of the kidney, which is made up of numerous nephrons. Its form varies in detail, but it always has at one end a device for receiving a filtrate of blood, and then a tubule that absorbs selected parts of the filtrate back into the bloodstream.

Nephrostome (nef ro stome) [Gr. nephros: kidney + stoma: opening] An opening in a nephridium through which body fluids can enter.

Nerve • A structure consisting of many neuronal axons and connective tissue.

Net primary production • Total photosynthesis minus respiration by plants.

Neural plate • A thickened strip of ectoderm along the dorsal side of the early vertebrate embryo; gives rise to the central nervous system.

Neural tube • An early stage in the development of the vertebrate nervous system consisting of a hollow tube created by two opposing folds of the dorsal ectoderm along the anterior-posterior body axis.

Neuromuscular junction • The region where a motor neuron contacts a muscle fiber, creating a synapse.

Neuron (noor' on) [Gr. neuron: nerve, sinew] • A cell derived from embryonic ectoderm and characterized by a membrane potential that can change in response to stimuli, generating action potentials. Action potentials are generated along an extension of the cell (the axon), which makes junctions (synapses) with other neurons, muscle cells, or gland cells.

Neurotransmitter • A substance, produced in and released by one neuron, that diffuses across a synapse and excites or inhibits the postsynaptic neuron.

Neurula (nure' you la) [Gr. neuron: nerve] • Embryonic stage during formation of the dorsal nerve cord by two ectodermal ridges.

Neutral allele • An allele that does not alter the functioning of the proteins for which it codes.

Neutral theory • A view of molecular evolution that postulates that most mutations do not affect the amino acid being coded for, and that such mutations accumulate in a population at rates driven by genetic drift and mutation rates.

Neutron (new' tron) [E.: neutral] • One of the three most fundamental particles of matter, with mass approximately 1 amu and no electrical charge.

Nicotinamide adenine dinucleotide • (See NAD.)

Nicotinamide adenine dinucleotide phosphate • (See NADP.)

Nitrification • The oxidation of ammonia to nitrite and nitrate ions, performed by certain soil bacteria.

Nitrogenase • In nitrogen-fixing organisms, an enzyme complex that mediates the stepwise reduction of atmospheric N₂ to ammonia.

Nitrogen fixation • Conversion of nitrogen gas to ammonia, which makes nitrogen available to living things. Carried out by certain prokaryotes, some of them free-living and others living within plant roots.

Node [L. nodus: knob, knot] • In plants, a (sometimes enlarged) point on a stem where a leaf is or was attached.

Node of Ranvier • A gap in the myelin sheath covering an axons, where the axonal membrane can fire action potentials.

Noncompetitive inhibitor • An inhibitor that binds the enzyme at a site other than the active site. (Contrast with competitive inhibitor.)

Nondisjunction • Failure of sister chromatids to separate in meiosis II or mitosis, or failure of homologous chromosomes to separate in meiosis I. Results in aneuploidy.

Nonpolar molecule • A molecule whose electric charge is evenly balanced from one end of the molecule to the other.

Nonsense (chain-terminating) mutation •

Mutations that change a codon for an amino acid to one of the codons (UAG, UAA, or UGA) that signal termination of translation. The resulting gene product is a shortened polypeptide that begins normally at the amino-terminal end and ends at the position of the altered codon. (Contrast with frame-shift mutation, missense mutation, synonymous mutation.)

Nonspecific defenses • Immunologic responses directed against most or all pathogens, generally without reference to the pathogens' antigens. These defenses include the skin, normal flora, lysozyme, the acidic stomach, interferon, and the inflammatory response.

Nonsynonymous mutation • A nucleotide substitution that changes the amino acid specified (i.e., AGC → AGA, or serine → arginine). (Compare with frame-shift mutation, missense mutation, nonsense mutation.)

Nonsynonymous substitution • The situation when a nonsynonymous mutation becomes widespread in a population. Typically influenced by natural selection. (Contrast with synonymous substitution.)

Nontracheophytes • Those plants lacking well-developed vascular tissue; the liverworts, hornworts, and mosses. (Contrast with tracheophytes.)

Normal flora • The bacteria and fungi that live on animal body surfaces without causing disease.

Norepinephrine • A neurotransmitter found in the central nervous system and also at the postganglionic nerve endings of the sympathetic nervous system. Also called noradrenaline.

Notochord (no' tow kord) [Gr. notos: back + chorde: string] • A flexible rod of gelatinous material serving as a support in the embryos of all chordates and in the adults of tunicates and lancelets.

Nuclear envelope • The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells.

Nucleic acid (new klay' ik) [E.; nucleus of a cell] • A long-chain alternating polymer of deoxyribose or ribose and phosphate groups, with nitrogenous bases—adenine, thymine, uracil, guanine, or cytosine (A, T, U, G, or C)—as side chains. DNA and RNA are nucleic acids.

Nucleoid (new' klee oid) • The region that harbors the chromosomes of a prokaryotic cell. Unlike the eukaryotic nucleus, it is not bounded by a membrane.

Nucleolar organizer (new klee' o lar) • A region on a chromosome that is associated with the formation of a new nucleolus following nuclear division. The site of the genes that code for ribosomal RNA.

Nucleolus (new klee' oh lus) [from L. diminutive of mix: little kernel or little nut] • A small, generally spherical body found within the nucleus of eukaryotic cells. The site of synthesis of ribosomal RNA.

Nucleoplasm (new' klee o plazm) • The fluid material within the nuclear envelope of a cell, as opposed to the chromosomes, nucleoli, and other particulate constituents.

Nucleosome • A portion of a eukaryotic chromosome, consisting of part of the DNA molecule wrapped around a group of histone molecules, and held together by another type of histone molecule. The chromosome is made up of many nucleosomes.

Nucleotide • The basic chemical unit (monomer) in a nucleic acid. A nucleotide in RNA consists of one of four nitrogenous bases linked to ribose, which in turn is linked to phosphate. In DNA, deoxyribose is present instead of ribose.

Nucleus (new' klee us) [from L. diminutive of mix: kernel or nut] • (1) In chemistry, the dense central portion of an atom, made up of protons and neutrons, with a positive charge. Surrounded by a cloud of negative-

GLOSSAR\

K charged electrons (2) In cells, the central-K located chamber of eukaryotic cells that is bounded by a double membrane and contains the chromosomes. The information center of the cell.

Null hypothesis • [the assertion that an effect proposed by its companion hypothesis does not in fact exist. **Nutrient** • \ food substance; or, in the case of mineral nutrients, an inorganic element required for completion of the life cycle of an organism

Oil • A triglyceride that is liquid at room temperature. (Contrast with fat.)

Okazaki fragments • Newly formed DNA strands making up the lagging strand in DNA replication. DNA ligase links the Okazaki fragments to give a continuous strand.

Olfactory • Having to do with the sense of smell.

Oligomer [Gr.: a few units] • A compound molecule of intermediate size, made up of two to a few monomers. (Contrast with monomer, polymer.)

Oligosaccharins • Plant hormones, derived from the plant cell wall, that trigger defenses against pathogens.

Ommatidium [Gr. omnia: an eye] • One of the units which, collected into groups of up to 20,000, make up the compound eye of arthropods.

Omnivore [L. omnis: all, everything + vorare: to devour] • An organism that eats both animal and plant material. (Contrast with carnivore, detritivore, herbivore.)

Oncogenic (ong' co jen' ik) [Gr. onkos: mass, tumor + genes: born] • Causing cancer.

Oocyte (oh' eh site) [Gr. oon: egg + kytos: cell] • The cell that gives rise to eggs in animals.

Oogenesis (oh' eh jen e sis) [Gr. oon: egg + genesis: source] • Female gametogenesis, leading to production of the egg.

Oogonium (oh' eh go' nee um) • In some algae and fungi, a cell in which an egg is produced.

Operator • The region of an operon that acts as the binding site for the repressor.

Operon • A genetic unit of transcription, typically consisting of several structural genes that are transcribed together; the operon contains at least two control regions: the promoter and the operator.

Opportunity cost • The sum of the benefits an animal forfeits by not being able to perform some other behavior during the time when it is performing a given behavior.

Opsin (op' sin) [Gr. opsis: sight] • The protein portion of the visual pigment rhodopsin. (See rhodopsin.)

Optic chiasm • Structure on the lower surface of the vertebrate brain where the two optic nerves come together.

Optical isomers • Isomers that differ in the configuration of the four different groups attached to a single carbon atom; so named

because solutions of the two isomers rotate the plane of polarized light in opposite directions. The two isomers are mirror images of one another.

Optimality models • Models developed to determine the structures or behaviors that best solve particular problems faced by organisms.

Order • In taxonomy, the category below the class and above the family; a group of related, similar families.

Organ • A body part, such as the heart, liver, brain, root, or leaf, composed of different tissues integrated to perform a distinct function for the body as a whole.

Organ identity genes • Plant genes that specify the various parts of the flower. See homeotic genes.

Organ of Corti • Structure in the inner ear that transforms mechanical forces produced from pressure waves ("sound waves") into action potentials that are sensed as sound.

Organelles (or' gan els) [L.: little organ] • Organized structures that are found in or on cells. Examples: ribosomes, nuclei, mitochondria, chloroplasts, cilia, and contractile vacuoles.

Organic • Pertaining to any aspect of living matter, e.g., to its evolution, structure, or chemistry. The term is also applied to any chemical compound that contains carbon.

Organism • Any living creature.

Organizer, embryonic • A region of an embryo which directs the development of nearby regions. In amphibian early gastrulas, the dorsal lip of the blastopore.

Origin of replication • A DNA sequence at which helicase unwinds the DNA double helix and DNA polymerase binds to initiate DNA replication.

Osmoregulation • Regulation of the chemical composition of the body fluids of an organism.

Osmoreceptor • A neuron that converts changes in the osmotic potential of interstitial fluids into action potentials.

Osmosis (oz mo' sis) [Gr. osmos: to push] • The movement of water through a differentially permeable membrane from one region to another where the water potential is more negative. This is often a region in which the concentration of dissolved molecules or ions is higher, although the effect of dissolved substances may be offset by hydrostatic pressure in cells with semi-rigid walls.

Ossicle (ah' sick ul) [L. os: bone] • The calcified construction unit of echinoderm skeletons.

Osteoblasts • Cells that lay down the protein matrix of bone.

Osteoclasts • Cells that dissolve bone.

Otolith (oh' tun lith) [Gk. otikos: ear + lithos: stone] • Structures in the vertebrate vestibular apparatus that mechanically stimulate hair cells when the head moves or changes position.

Outgroup • A taxon that separated from another taxon, whose lineage is to be inferred, before the latter underwent evolutionary radiation.

Oval window • The flexible membrane which, when moved by the bones of the middle ear, produces pressure waves in the inner ear

Ovary (oh' var ee) • Any female organ, in plants or animals, that produces an egg.

Oviduct [L. ovum: egg + ducere: to lead] • In mammals, the tube serving to transport eggs to the uterus or to outside of the body.

Oviparous (oh vip' uh rus) • Reproduction in which eggs are released by the female and development is external to the mother's body. (Contrast with viviparous.)

Ovulation • The release of an egg from an ovary.

Ovule (oh' vule) [L. ovulum: little egg] • In plants, an organ that contains a gameto-phyte and, within the gametophyte, an egg; when it matures, an ovule becomes a seed.

Ovum (oh' vum) [L.: egg] • The egg, the female sex cell.

Oxidation (ox i day' shun) • Relative loss of electrons in a chemical reaction; either outright removal to form an ion, or the sharing of electrons with substances having a greater affinity for them, such as oxygen. Most oxidation, including biological ones, are associated with the liberation of energy. (Contrast with reduction.)

Oxidative phosphorylation • ATP formation in the mitochondrion, associated with flow of electrons through the respiratory chain.

Oxidizing agent • A substance that can accept electrons from another. The oxidizing agent becomes reduced; its partner becomes oxidized.

P generation • Also called the parental generation. The individuals that mate in a genetic cross. Their immediate offspring are

the F₂ generation.

Pacemaker • That part of the heart which undergoes most rapid spontaneous contraction, thus setting the pace for the beat of the entire heart. In mammals, the sinoatrial (SA) node. Also, an artificial device, implanted in the heart, that initiates rhythmic contraction of the organ.

Pacinian corpuscle • A sensory neuron surrounded by sheaths of connective tissue. Found in the deep layers of the skin, where it senses touch and vibration.

Pair rule genes • Segmentation genes that divide the *Drosophila* larva into two segments each.

Paleomagnetism • The record of the changing direction of Earth's magnetic field as stored in lava flows. Used to accurately date extremely ancient events.

Paleontology (pale' ee on tol' oh jee) [Gr. palaios: ancient, old + logos: discourse] • The scientific study of fossils and all aspects of extinct life.

Pancreas (pan' cree us) • A gland, located near the stomach of vertebrates, that secretes digestive enzymes into the small

GLOSSARY

intestine and releases insulin into the bloodstream.

Pangaea (pan jee' uh) [Gk. pan: all, every] • The single land mass formed when all the continents came together in the Permian period. (Contrast with Gondwana.)

Parabronchi • Passages in the lungs of birds through which air flows.

Paradigm • A general framework within which a scientific or philosophical discipline is viewed and within which questions are asked and hypotheses are developed. Scientific revolutions usually invoke major paradigm changes. (Contrast with hypothesis, theory.)

Parallel evolution • Evolutionary patterns that exist in more than one lineage. Often the result of underlying developmental processes.

Parapatric speciation [Gr. para: beside + patria: fatherland] • Development of reproductive isolation when the barrier is not geographic but is a difference in some other physical condition (such as soil nutrient content) that prevents gene flow between the subpopulations. (Contrast with allopatric speciation, sympatric speciation.)

Paraphyletic taxon • A taxon that includes some, but not all, of the descendants of a single ancestor.

Parasite • An organism that attacks and consumes parts of an organism much larger than itself. Parasites sometimes, but not always, kill the host.

Parasitoid • A parasite that is so large relative to its host that only one individual or at most a few individuals can live within a single host.

Parasympathetic nervous system • A portion of the autonomic (involuntary) nervous system. Activity in the parasympathetic nervous system produces effects such as decreased blood pressure and decelerated heart beat. (Contrast with sympathetic nervous system.)

Parathormone • Hormone secreted by the parathyroid glands. Stimulates osteoclast activity and raises blood calcium levels.

Parathyroids • Four glands on the posterior surface of the thyroid that produce and release parathormone.

Parenchyma (pair eng' kyma) [Gr. para: beside + enchyma: infusion] • A plant tissue composed of relatively unspecialized cells without secondary walls.

Parental investment • Investment in one offspring or group of offspring that reduces the ability of the parent to assist other offspring.

Parsimony • The principle of preferring the simplest among a set of plausible explanations of a phenomenon. Commonly employed in evolutionary and biogeographic studies.

Parthenocarpy • Formation of fruit from a flower without fertilization.

Parthenogenesis (par' then oh jen' e sis) [Gr. parthenos: virgin + genesis: source] • The production of an organism from an unfertilized egg.

Partial pressure • The portion of the barometric pressure of a mixture of gases that is due to one component of that mixture. For example, the partial pressure of oxygen at sea level is 20.9% of barometric pressure.

Patch clamping • A technique for isolating a tiny patch of membrane to allow the study of ion movement through a particular channel.

Pathogen (path' o jen) [Gr. pathos: suffering + gignomai: causing] • An organism that causes disease.

Pattern formation • In animal embryonic development, the organization of differentiated tissues into specific structures such as wings.

Pedigree • The pattern of transmission of a genetic trait in a family.

Pelagic zone (puh ladj' ik) [Gr. pelagos: the sea] • The open waters of the ocean.

Penetrance • Of a genotype, the proportion of individuals with that genotype who show the expected phenotype.

PEP carboxylase • The enzyme that combines carbon dioxide with PEP to form a 4-carbon dicarboxylic acid at the start of C₄ photosynthesis or of Crassulacean acid metabolism (CAM).

Pepsin [Gr. pepsis: digestion] • An enzyme, in gastric juice, that digests protein.

Peptide linkage • The connecting group in a protein chain, -CO-NH-, formed by removal of water during the linking of amino acids, -COOH to -NH-,.

Peptidoglycan • The cell wall material of many prokaryotes, consisting of a single enormous molecule that surrounds the entire cell.

Perennial (per ren' ee al) [L. per. through + annus: a year] • Referring to a plant that lives from year to year. (Contrast with annual, biennial.)

Perfect flower • A flower with both stamens and carpels, therefore hermaphroditic.

Pericycle [Gr. peri: around + kyklos: ring or circle] • In plant roots, tissue just within the endodermis, but outside of the root vascular tissue. Meristematic activity of pericycle cells produces lateral root primordia.

Periderm • The outer tissue of the secondary plant body, consisting primarily of cork.

Period • (1) A minor category in the geological time scale. (2) The duration of a cyclical event, such as a circadian rhythm.

Peripheral nervous system • Neurons that transmit information to and from the central nervous system and whose cell bodies reside outside the brain or spinal cord.

Peristalsis (pair' i stall' sis) [Gr. peri: around + stellein: place] • Wavelike muscular contractions proceeding along a tubular organ, propelling the contents along the tube.

Peritoneum • The mesodermal lining of the coelom among coelomate animals.

Permease • A membrane protein that specifically transports a compound or family of compounds across the membrane.

Peroxisome • An organelle that houses reactions in which toxic peroxides are formed. The peroxisome isolates these peroxides from the rest of the cell.

Petal • In an angiosperm flower, a sterile modified leaf, nonphotosynthetic, frequently brightly colored, and often serving to attract pollinating insects.

Petiole (pet' ee ole) [L. ptiolus: small foot] • The stalk of a leaf.

pH • The negative logarithm of the hydrogen ion concentration; a measure of the acidity of a solution. A solution with pH = 7 is said to be neutral; pH values higher than 7 characterize basic solutions, while acidic solutions have pH values less than 7.

Phage (fayj) • Short for bacteriophage.

Phagocyte • A white blood cell that ingests microorganisms by endocytosis.

Phagocytosis [Gr.: phagein to eat; cell-eating] • A form of endocytosis, the uptake of a solid particle by forming a pocket of plasma membrane around the particle and pinching off the pocket to form an intracellular particle bounded by membrane. (Contrast with pinocytosis.)

Pharynx [Gr.: throat] • The part of the gut between the mouth and the esophagus.

Phenotype (fee' no type) [Gr. phanein: to show] • The observable properties of an individual as they have developed under the combined influences of the genetic constitution of the individual and the effects of environmental factors. (Contrast with genotype.)

Phenotypic plasticity • The fact that the phenotype of an organism is determined by a complex series of developmental processes that are affected by both its genotype and its environment.

Pheromone (feer' o mone) [Gr. phew: carry + hormon: excite, arouse] • A chemical substance used in communication between organisms of the same species.

Phloem (flo' um) [Gr. phloos: bark] • In vascular plants, the food-conducting tissue. It consists of sieve cells or sieve tubes, fibers, and other specialized cells.

Phosphate group • The functional group -OPO₃FT; the transfer of energy from one compound to another is often accomplished by the transfer of a phosphate group.

Phosphodiester linkage • The connection in a nucleic acid strand, formed by linking two nucleotides.

Phospholipids • Cellular materials that contain phosphorus and are soluble in organic solvents. An example is lecithin (phosphatidyl choline). Phospholipids are important constituents of cellular membranes. (See lipids.)

Phosphorylation • The addition of a phosphate group.

Photoautotroph • An organism that obtains energy from light and carbon from carbon

GLOSSARY

dioxide. (Contrast with chemoautotroph, chemoheterotroph, photoheterotroph.)

Photoheterotroph • An organism that obtains energy from light but must obtain it-- carbon from organic compounds. (Contrast with chemoautotroph, chemo-heterotroph, photoautotroph.)

Photon (foe' tohn) [Gr. photos: light] • A quantum of visible radiation; a "packet" of light energy

Photoperiod (foe* tow peer' ee ud) • The duration of a period of light, such as the length of time in a 24-hour cycle in which daylight is present. The regulation of processes such as flowering by the changing length of day (or of night) is known as photoperiodism.

Photoreceptor • (1) A protein (pigment) that triggers a physiological response when it absorbs a photon. (2) A cell that senses and responds to light energy.

Photorespiration • Light-driven uptake of oxygen and release of carbon dioxide, the carbon being derived from the early reactions of photosynthesis.

Photosynthesis (foe tow sin' the sis) [literal-lv, "synthesis out of light"] • Metabolic processes, carried out by green plants, by which visible light is trapped and the energy used to synthesize compounds such as ATP and glucose.

Phototropin • A yellow protein that is the photoreceptor responsible for phototropism.

Phototropism [Gr. photos: light + trope: a turning] • A directed plant growth response to light.

Phylogenetic tree • Graphic representation of lines of descent among organisms.

Phylogeny (fy loj' e nee) [Gr. phylon: tribe, race + genesis: source] • The evolutionary history of a particular group of organisms; also, the diagram of the "family tree" that shows genetic linkages between ancestors and descendants.

Phylum (plural: phyla) [Gr. phylon: tribe, stock] • In taxonomy, a high-level category just beneath kingdom and above the class; a group of related, similar classes.

Physiology (fiz' ee ol' o jee) [Gr. physis: natural form + logos: discourse, study] • The scientific study of the functions of living organisms and the individual organs, tissues, and cells of which they are composed.

Phytoalexins • Substances toxic to fungi, produced by plants in response to fungal infection.

Phytochrome (fy' tow krome) [Gr. phyton: plant + chroma: color] • A plant pigment regulating a large number of developmental and other phenomena in plants; can exist in two different forms, one of which is active and the other is not. Different wavelengths of light can drive it from one form to the other.

Phytoplankton (fy' tow plangk' ton) [Gr. phyton: plant + planktos: wandering] • The autotrophic portion of the plankton, consisting mostly of algae.

Pigment • A substance that absorbs visible light.

Pilus (pill' us) [Lat. pilus: hair] • A surface appendage by which some bacteria adhere to one another during conjugation.

Pinocytosis [Gr.: drinking cell] • A form of endocytosis; the uptake of liquids by engulfing a sample of the external medium into a pocket of the plasma membrane followed by pinching off the pocket to form an intracellular vesicle. (Contrast with phagocytosis and endocytosis.)

Pistil [L. pistillum: pestle] • The female structure of an angiosperm flower, within which the ovules are borne. May consist of a single carpel, or of several carpels fused into a single structure. Usually differentiated into ovary, style, and stigma.

Pith • In plants, relatively unspecialized tissue found within a cylinder of vascular tissue.

Pituitary • A small gland attached to the base of the brain in vertebrates. Its hormones control the activities of other glands.

Also known as the hypophysis.

Placenta (pla sen' ta) [Gr. plax: flat surface] • The organ found in most mammals that provides for the nourishment of the fetus and elimination of the fetal waste products.

Placental (pla sen' tal) • Pertaining to mammals of the subclass Eutheria, a group characterized by the presence of a placenta; contains the majority of living species of mammals.

Plankton [Gr. planktos: wandering] • The free-floating organisms of the sea and fresh water that for the most part move passively with the water currents. Consisting mostly of microorganisms and small plants and animals. (Contrast with nekton.)

Plant • A member of the kingdom Plantae. Multicellular, gaining its nutrition by photosynthesis.

Planula (plan' yew la) [L. planum: something flat] • The free-swimming, ciliated larva of the cnidarians.

Plaque (plack) [Fr.: a metal plate or coin] • (1) A circular clearing in a turbid layer (lawn) of bacteria growing on the surface of a nutrient agar gel. Produced by successive rounds of infection initiated by a single bacteriophage. (2) An accumulation of prokaryotic organisms on tooth enamel. Acids produced by the metabolism of these microorganisms can cause tooth decay.

Plasma (plaz' muh) [Gr. plassein: to mold] • The liquid portion of blood, in which blood cells and other particulates are suspended.

Plasma cell • An antibody-secreting cell that developed from a B cell. The effector cell of the humoral immune system.

Plasma membrane • The membrane that surrounds the cell, regulating the entry and exit of molecules and ions. Every cell has a plasma membrane.

Plasmid • A DNA molecule distinct from the chromosome(s); that is, an extrachromosomal element. May replicate independently of the chromosome.

Plasmodesma (plural: plasmodesmata) [Gr. plasma: formed or molded + desmos: band] • A cytoplasmic strand connecting two adjacent plant cells.

Plasmolysis (plaz mol' i sis) • Shrinking of the cytoplasm and plasma membrane away from the cell wall, resulting from the osmotic outflow of water. Occurs only in cells with rigid cell walls.

Plastid • Organelle in plants that serves for food manufacture (by photosynthesis) or food storage; bounded by a double membrane.

Platelet • A membrane-bounded body without a nucleus, arising as a fragment of a cell in the bone marrow of mammals. Important to blood-clotting action.

Pleiotropy (plee' a tro pee) [Gr. pleion: more] • The determination of more than one character by a single gene.

Pleural membrane [Gk. pleuras: rib, side] • The membrane lining the outside of the lungs and the walls of the thoracic cavity. Inflammation of these membranes is a condition known as pleurisy.

Podocytes • Cells of Bowman's capsule of the nephron that cover the capillaries of the glomerulus, forming filtration slits.

Poikilotherm (poy' kill o therm) [Gr. poikilos: varied + therme: heat] • An animal whose body temperature tends to vary with the surrounding environment. (Contrast with homeotherm, heterotherm.)

Point mutation • A mutation that results from a small, localized alteration in the chemical structure of a gene. Such mutations can give rise to wild-type revertants as a result of reverse mutation. In genetic crosses, a point mutation behaves as if it resided at a single point on the genetic map. (Contrast with deletion.)

Polar body • A nonfunctional nucleus produced by meiosis, accompanied by very little cytoplasm. The meiosis which produces the mammalian egg produces in addition three polar bodies.

Polar molecule • A molecule in which the electric charge is not distributed evenly in the covalent bonds.

Polarity • In development, the difference between one end and the other. In chemistry, the property that makes a polar molecule.

Pollen [L.: fine powder, dust] • The fertilizing element of seed plants, containing the male gametophyte and the gamete, at the stage in which it is shed.

Pollination • Process of transferring pollen from the anther to the receptive surface (stigma) of the ovary in plants.

Poly- [Gr. poly: many] • A prefix denoting multiple entities.

Polygamy [Gr. poly: many + gamos: marriage] • A breeding system in which an individual acquires more than one mate. In polyandry, a female mates with more than one male, in polygyny, a male mates with more than one female.

GLOSSARY

Polygenes • Multiple loci whose alleles increase or decrease a continuously variable phenotypic trait.

Polymer • A large molecule made up of similar or identical subunits called monomers. (Contrast with monomer, oligomer.)

Polymerase chain reaction (PCR) • A technique for the rapid production of millions of copies of a particular stretch of DNA.

Polymerization reactions • Chemical reactions that generate polymers by means of condensation reactions.

Polymorphism (pol' lee mor' fiz um) [Gr. poll/: many + morphe: form, shape] • (1) In genetics, the coexistence in the same population of two distinct hereditary types based on different alleles. (2) In social organisms such as colonial cnidarians and social insects, the coexistence of two or more functionally different castes within the same colony.

Polyp • The sessile, asexual stage in the life cycle of most cnidarians.

Polypeptide • A large molecule made up of many amino acids joined by peptide linkages. Large polypeptides are called proteins.

Polyphyletic group • A group containing taxa, not all of which share the most recent common ancestor.

Polyploid (pol' lee ploid) • A cell or an organism in which the number of complete sets of chromosomes is greater than two.

Polysaccharide • A macromolecule composed of many monosaccharides (simple sugars). Common examples are cellulose and starch.

Polysome • A complex consisting of a threadlike molecule of messenger RNA and several (or many) ribosomes. The ribosomes move along the mRNA, synthesizing polypeptide chains as they proceed.

Polytene (pol' lee teen) [Gr. poly, many + taenia: ribbon] • An adjective describing giant interphase chromosomes, such as those found in the salivary glands of fly larvae. The characteristic, reproducible pattern of bands and bulges seen on these chromosomes has provided a method for preparing detailed chromosome maps of several organisms.

Pons [L. pons: bridge] • Region of the brain stem anterior to the medulla.

Population • Any group of organisms coexisting at the same time and in the same place and capable of interbreeding with one another.

Population density • The number of individuals (or modules) of a population in a unit of area or volume.

Population genetics • The study of genetic variation and its causes within populations.

Population structure • The proportions of individuals in a population belonging to different age classes (age structure). Also, the distribution of the population in space.

Portal vein • A vein connecting two capillary beds, as in the hepatic portal system.

Positive control • The situation in which a regulatory macromolecule is needed to turn transcription of structural genes on. In its absence, transcription will not occur.

Positive cooperativity • Occurs when a molecule can bind several ligands and each one that binds alters the conformation of the molecule so that it can bind the next ligand more easily. The binding of four molecules of O₂ by hemoglobin is an example of positive cooperativity.

Postabsorptive period • When there is no food in the gut and no nutrients are being absorbed.

Postsynaptic cell • The cell whose membranes receive the neurotransmitter released at a synapse.

Predator • An organism that kills and eats other organisms. Predation is usually thought of as involving the consumption of animals by animals, but it can also mean the eating of plants.

Presynaptic excitation/inhibition • Occurs when a neuron modifies activity at a synapse by releasing a neurotransmitter onto the presynaptic nerve terminal.

Prey [L. praeda: booty] • An organism consumed as an energy source.

Primary active transport • Form of active transport in which ATP is hydrolyzed, yielding the energy required to transport ions against their concentration gradients. (Contrast with secondary active transport.)

Primary growth • In plants, growth produced by the apical meristems. (Contrast with secondary growth.)

Primary producer • A photosynthetic or chemosynthetic organism that synthesizes complex organic molecules from simple inorganic ones.

Primary succession • Succession that begins in an area initially devoid of life, such as on recently exposed glacial till or lava flows.

Primary structure • The specific sequence of amino acids in a protein.

Primary wall • Cellulose-rich cell wall layers laid down by a growing plant cell.

Primate (pry' mate) • A member of the order Primates, such as a lemur, monkey, ape, or human.

Primer • A short, single-stranded segment of DNA serving as the necessary starting material for the synthesis of a new DNA strand, which is synthesized from the 3' end of the primer.

Primitive streak • A line running axially along the blastodisc, the site of inward cell migration during formation of the three-layered embryo. Formed in the embryos of birds and fish.

Primordium [L. primordiū: origin] • The most rudimentary stage of an organ or other part.

Principle of continuity • States that because life probably evolved from nonlife by a continuous, gradual process, all postulated stages in the evolution of life should be derivable from preexisting states. (Compare with signature principle.)

Pro- [L.: first, before, favoring] • A prefix often used in biology to denote a developmental stage that comes first or an evolutionary form that appeared earlier than another. For example, prokaryote, prophase.

Probe • A segment of single stranded nucleic acid used to identify DNA molecules containing the complementary sequence.

Procambium • Primary meristem that produces the vascular tissue.

Progesterone [L. pro: favoring + gestare: to bear] • A vertebrate female sex hormone that maintains pregnancy.

Prokaryotes (pro kar' ry otes) [L. pro: before + Gk. karyon: kernel, nucleus] • Organisms whose genetic material is not contained within a nucleus. The bacteria. Considered an earlier stage in the evolution of life than the eukaryotes.

Prometaphase • The phase of nuclear division that begins with the disintegration of the nuclear envelope.

Promoter • The region of an operon that acts as the initial binding site for RNA polymerase.

Proofreading • The correction of an error in DNA replication just after an incorrectly paired base is added to the growing polynucleotide chain.

Prophage (pro' fayj) • The noninfectious units that are linked with the chromosomes of the host bacteria and multiply with them but do not cause dissolution of the cell. Prophage can later enter into the lytic phase to complete the virus life cycle.

Prophase (pro' phase) • The first stage of nuclear division, during which chromosomes condense from diffuse, threadlike material to discrete, compact bodies.

Prostaglandin • Any one of a group of specialized lipids with hormone-like functions. It is not clear that they act at any considerable distance from the site of their production.

Prosthetic group • Any nonprotein portion of an enzyme.

Protease (pro' tee ase) • See proteolytic enzyme.

Protein (pro' teen) [Gr. protos: first] • One of the most fundamental building substances of living organisms. A long-chain polymer of amino acids with twenty different common side chains. Occurs with its polymer chain extended in fibrous proteins, or coiled into a compact macromolecule in enzymes and other globular proteins.

Proteolytic enzyme • An enzyme whose main catalytic function is the digestion of a protein or polypeptide chain. The digestive enzymes trypsin, pepsin, and carboxypeptidase are all proteolytic enzymes (proteases).

Protist • Those eukaryotes not included in the kingdoms Animalia, Fungi, or Plantae.

Protobiont • Aggregates of abiotically produced molecules that cannot reproduce but do maintain internal chemical environments that differ from their surroundings.

Protoderm • Primary meristem that gives rise to epidermis.

Proton (pro' tan) [Gr. protos: first] • One of the three most fundamental particles of matter, with mass approximately 1 amu and an electrical charge of $+1$.

Proto-oncogenes • The normal alleles of genes possessing oncogenes (cancer-causing genes). Proto-oncogenes encode growth factors and receptor proteins.

Protostome • One of the major lineages of animal evolution. Characterized by spiral, determinate cleavage of the egg, and by schizocoelous development. (Compare with deuterostome.)

Prototroph (pro' tow trofe') [Gr. protos: first + tropic: to nourish] • The nutritional wild type, or reference form, of an organism. Any deviant form that requires growth nutrients not required by the prototrophic form is said to be a nutritional mutant, or auxotroph.

Protozoa • A group of single-celled organisms classified by some biologists as a single phylum; includes the flagellates, amoebas, and ciliates. This textbook follows most modern classifications in elevating the protozoans to a distinct kingdom (Protista) and each of their major subgroups to the rank of phylum.

Proximal • Near the point of attachment or other reference point. (Contrast with distal.)

Pseudocoelom • A body cavity not surrounded by a peritoneum. Characteristic of nematodes and rotifers.

Pseudogene • A DNA segment that is homologous to a functional gene but contains a nucleotide change that prevents its expression.

Pseudoplasmodium [Gr. *pseudes*: false + *plasma*: mold or form] • In the cellular slime molds such as *Dictyostelium*, an aggregation of single amoeboid cells. Occurs prior to formation of a fruiting structure.

Pseudopod (soo' do pod) [Gr. *pseudes*: false + *podos*: foot] • A temporary, soft extension of the cell body that is used in location, attachment to surfaces, or engulfing particles.

Pulmonary • Pertaining to the lungs.

Punctuated equilibrium • An evolutionary pattern in which periods of rapid change are separated by longer periods of little or no change.

Pupa (pew' pa) [L: doll, puppet] • In certain insects (the Holometabola), the encased developmental stage that intervenes between the larva and the adult.

Pupil • The opening in the vertebrate eye through which light passes.

Purine (pure' een) • A type of nitrogenous base. The purines adenine and guanine are found in nucleic acids.

Purkinje fibers • Specialized heart muscle cells that conduct excitation throughout the ventricular muscle.

Pyramid of biomass • Graphical representation of the total body masses at different trophic levels in an ecosystem.

Pyramid of energy • Graphical representation of the total energy contents at different trophic levels in an ecosystem.

Pyrimidine (peer im' a deen) • A type of nitrogenous base. The pyrimidines cytosine, thymine, and uracil are found in nucleic acids.

Pyruvate • A three-carbon acid; the end product of glycolysis and the raw material for the citric acid cycle.

Q₁₀ • A value that compares the rate of a biochemical process or reaction over a 10°C range of temperature. A process that is not temperature-sensitive has a Q₁₀ of 1. Values of 2 or 3 mean the reaction speeds up as temperature increases.

Quantum (kwon' turn) [L. *quantus*: how great] • An indivisible unit of energy.

Quaternary structure • Of aggregating proteins, the arrangement of polypeptide sub-units.

R factor (resistance factor) • A plasmid that contains one or more genes that encode resistance to antibiotics.

Radial symmetry • The condition in which two halves of a body are mirror images of each other regardless of the angle of the cut, providing the cut is made along the center line. Thus, a cylinder cut lengthwise down its center displays this form of symmetry. (Contrast with biradial symmetry.)

Radioisotope • A radioactive isotope of an element. Examples are carbon-14 (¹⁴C) and hydrogen-3, or tritium (³H).

Radiometry • The use of the regular, known rates of decay of radioisotopes of elements to determine dates of events in the distant past.

Rain shadow • A region of low precipitation on the leeward side of a mountain range.

Ramet • The repeated morphological units of sessile, modular organisms. (Contrast with genet.)

Random genetic drift • Evolution (change in gene proportions) by chance processes alone.

Rate constant • Of a particular chemical reaction, a constant which, when multiplied by the concentration(s) of reactant(s), gives the rate of the reaction.

Reactant • A chemical substance that enters into a chemical reaction with another substance.

Reaction, chemical • A process in which atoms combine or change bonding partners.

Realized niche • The actual niche occupied by an organism; it differs from the fundamental niche because of the presence of other species.

Receptive field • Of a neuron, the area on the retina from which the activity of that neuron can be influenced.

Receptor potential • The change in the resting potential of a sensory cell when it is stimulated.

Recessive • See dominance.

Reciprocal altruism • The exchange of altruistic acts between two or more individuals. The acts may be separated considerably in time.

Reciprocal crosses • A pair of crosses, in one of which a female of genotype A mates with a male of genotype B and in the other of which a female of genotype B mates with a male of genotype A.

Recognition site (also called a restriction site) • A sequence of nucleotides in DNA to which a restriction enzyme binds and then cuts the DNA.

Recombinant • An individual, meiotic product, or single chromosome in which genetic materials originally present in two individuals end up in the same haploid complement of genes. The reshuffling of genes can be either by independent segregation, or by crossing over between homologous chromosomes. For example, a human may pass on genes from both parents in a single haploid gamete.

Recombinant DNA technology • The

application of genetic tools (restriction endonucleases, plasmids, and transformation) to the production of specific proteins by biological "factories" such as bacteria.

Rectum • The terminal portion of the gut, ending at the anus.

Redox reaction • A chemical reaction in which one reactant becomes oxidized and the other becomes reduced.

Reducing agent • A substance that can donate electrons to another substance. The reducing agent becomes oxidized, and its partner becomes reduced.

Reduction (re duk' shun) • Gain of electrons; the reverse of oxidation. Most reductions lead to the storage of chemical energy, which can be released later by an oxidation reaction. Energy storage compounds such as sugars and fats are highly reduced compounds. (Contrast with oxidation.)

Reflex • An automatic action, involving only a few neurons (in vertebrates, often in the spinal cord), in which a motor response swiftly follows a sensory stimulus.

Refractory period • Of a neuron, the time interval after an action potential, during which another action potential cannot be elicited.

Regulative development • A pattern of animal embryonic development in which the fates of the first blastomeres are not absolutely fixed. (Contrast with mosaic development.)

Regulatory gene • A gene that contains the information for making a regulatory macro-molecule, often a repressor protein.

Releaser • A sensory stimulus that triggers a fixed action pattern.

Releasing hormone • One of several hypothalamic hormones that stimulates the secretion of anterior pituitary hormone.

REM sleep • A sleep state characterized by dreaming, skeletal muscle relaxation, and rapid eye movements.

Renal [L. renes: kidneys] • Relating to the kidneys.

Replication fork • A point at which a DNA molecule is replicating. The fork forms by the unwinding of the parent molecule.

Repressible enzyme • An enzyme whose synthesis can be decreased or prevented by

GLOSSARY

the presence of a particular compound. A repressible operon often controls the synthesis of such an enzyme.

Repressor • A protein coded by the regulatory gene. The repressor can bind to a specific operator and prevent transcription of the operon.

Reproductive isolating mechanism • Any trait that prevents individuals from two different populations from producing fertile hybrids.

Reproductive isolation • The condition in which a population is not exchanging genes with other populations of the same

species.

Resolving power • Of an optical device such as a microscope, the smallest distance between two lines that allows the lines to be seen as separate from one another.

Resource • Something in the environment required by an organism for its maintenance and growth that is consumed in the process of being used.

Resource defense polygamy • A breeding system in which individuals of one sex (usually males) defend resources that are attractive to individuals of the other sex (usually females); individuals holding better resources attract more mates.

Respiration (res pi ra' shun) [L. spvrare: to breathe] • (1) Cellular respiration; the oxidation of the end products of glycolysis with the storage of much energy in ATP. The oxidant in the respiration of eukaryotes is oxygen gas. Some bacteria can use nitrate or sulfate instead of O_2 - (2) Breathing.

Respiratory chain • The terminal reactions of cellular respiration, in which electrons are passed from NAD or FAD, through a series of intermediate carriers, to molecular oxygen, with the concomitant production of ATP.

Resting potential • The membrane potential of a living cell at rest. In cells at rest, the interior is negative to the exterior. (Contrast with action potential, electrotonic potential.)

Restoration ecology • The science and practice of restoring damaged or degraded ecosystems.

Restriction endonuclease • Any one of several enzymes, produced by bacteria, that break foreign DNA molecules at very specific sites. Some produce "sticky ends." Extensively used in recombinant DNA technology.

Restriction map • A partial genetic map of a DNA molecule, showing the points at which particular restriction endonuclease recognition sites reside.

Reticular system • A central region of the vertebrate brain stem that includes complex fiber tracts conveying neural signals between the forebrain and the spinal cord, with collateral fibers to a variety of nuclei that are involved in autonomic functions, including arousal from sleep.

Retina (rett' in uh) [L. rete: net] • The light-sensitive layer of cells in the vertebrate or cephalopod eye.

Retinal • The light-absorbing portion of visual pigment molecules. Derived from (J-carotene).

Retrovirus • An RNA virus that contains reverse transcriptase. Its RNA serves as a template for cDNA production, and the cDNA is integrated into a chromosome of the mammalian host cell.

Reverse transcriptase • An enzyme that catalyzes the production of DNA (cDNA), using RNA as a template; essential to the reproduction of retroviruses.

RFLP (Restriction fragment length polymorphism) • Coexistence of two or more patterns of restriction fragments (patterns produced by restriction enzymes), as revealed by a probe. The polymorphism reflects a difference in DNA sequence on homologous chromosomes.

Rhizoids (rye' zoids) [Gr. rhiza: root] • Hairlike extensions of cells in mosses, liverworts, and a few vascular plants that serve the same function as roots and root hairs in vascular plants. The term is also applied to branched, rootlike extensions of some fungi and algae.

Rhizome (rye' zome) [Gr. rhizoma: mass of roots] • A special underground stem (as opposed to root) that runs horizontally beneath the ground.

Rhodopsin • A photopigment used in the visual process of transducing photons of light into changes in the membrane potential of photoreceptor cells.

Ribonucleic acid • See RNA.

Ribosomal RNA (rRNA) • Several species of RNA that are incorporated into the ribosome. Involved in peptide bond formation.

Ribosome • A small organelle that is the site of protein synthesis.

Ribozyme • An RNA molecule with catalytic activity.

Ribulose 1,5-bisphosphate (RuBP) • The compound in chloroplasts which reacts with carbon dioxide in the first reaction of the Calvin-Benson cycle.

Risk cost • The increased chance of being injured or killed as a result of performing a behavior, compared to resting.

RNA (ribonucleic acid) • A nucleic acid using ribose. Various classes of RNA are involved in the transcription and translation of genetic information. RNA serves as the genetic storage material in some viruses.

RNA polymerase • An enzyme that catalyzes the formation of RNA from a DNA template.

RNA splicing • The last stage of RNA processing in eukaryotes, in which the transcripts of introns are excised through the action of small nuclear ribonucleoprotein particles (snRNP).

Rods • Light-sensitive cells (photoreceptors) in the retina. (Contrast with cones.)

Root cap • A thimble-shaped mass of cells, produced by the root apical meristem, that protects the meristem and that is the organ that perceives the gravitational stimulus in root gravitropism.

Root hair • A specialized epidermal cell with a long, thin process that absorbs water and minerals from the soil solution.

rRNA • See ribosomal RNA.

Rubisco (RuBP carboxylase) • Enzyme that combines carbon dioxide with ribulose bis-phosphate to produce 3-phosphoglycerate, the first product of C₃ photosynthesis. The most abundant protein on Earth.

Rumen (rew' mun) • The first division of the ruminant stomach. It stores and initiates bacterial fermentation of food. Food is regurgitated from the rumen for further chewing.

Ruminant • An herbivorous, cud-chewing mammal such as a cow, sheep, or deer, having a stomach consisting of four compartments.

S phase • In the cell cycle, the stage of interphase during which DNA is replicated. (Contrast with G¹ phase, G₂ phase.)

Saprobe [Gr. sapos: rotten + bios: life] • An organism (usually a bacterium or fungus) that obtains its carbon and energy directly from dead organic matter.

Sarcomere (sark' o meer) [Gr. sark: flesh + mews: a part] • The contractile unit of a skeletal muscle.

Saturated hydrocarbon • A compound consisting only of carbon and hydrogen, with the hydrogen atoms connected by single bonds.

Schizocoelous development • Formation of a coelom during embryological development by a splitting of mesodermal masses.

Schwann cell • A glial cell that wraps around part of the axon of a peripheral neuron, creating a myelin sheath.

Sclereid [Gr. skleros: hard] • A type of sclerenchyma cell, commonly found in nutshells, that is not elongated.

Sclerenchyma (skier eng' kyma) [Gr. skleros: hard + kyinus, juice] • A plant tissue composed of cells with heavily thickened cell walls, dead at functional maturity. The principal types of sclerenchyma cells are fibers and sclereids.

Secondary active transport • Form of active transport in which ions or molecules are transported against their concentration gradient using energy obtained by relaxation of a gradient of sodium ion concentration rather than directly from ATP. (Contrast with primary active transport.)

Secondary compound • A compound synthesized by a plant that is not needed for basic cellular metabolism. Typically has an antiherbivore or antiparasite function.

Secondary growth • In plants, growth produced by vascular and cork cambia, contributing to an increase in girth. (Contrast with primary growth.)

Secondary structure • Of a protein, localized regularities of structure, such as the α helix and the β pleated sheet.

Secondary succession • Ecological succession after a disturbance that does not elimi-

SS \K>

nate all the organisms that originally lived on the site

Secondary wall • Wall layers laid down by a plant cell that has ceased growing; often impregnated with lignin or suberin.

Second law of thermodynamics • States that in any real (irreversible) process, there is a decrease in free energy and an increase in entropy.

Second messenger • A compound, such as cyclic AMP, that is released within a target cell after a hormone or other "first messenger" has bound to a surface receptor on a cell; the second messenger triggers further reactions within the cell.

Secretin (si kreet' in) • A peptide hormone secreted by the upper region of the small intestine when acidic chyme is present. Stimulates the pancreatic duct to secrete bicarbonate ions.

Section • A thin slice, usually for microscopy, as a tangential section or a transverse section.

Seed • A fertilized, ripened ovule of a gymnosperm or angiosperm. Consists of the embryo, nutritive tissue, and a seed coat.

Seed crop • The number of seeds produced by a plant during a particular bout of reproduction.

Seedling • A young plant that has grown from a seed (rather than by grafting or by other means.)

Segmentation genes • In insect larvae, genes that determine the number and polarity of larval segments.

Segment polarity genes • Genes that determine the boundaries and front-to-back organization of the segments in the *Drosophila* larva.

Segregation (genetic) • The separation of alleles, or of homologous chromosomes, from one another during meiosis so that each of the haploid daughter nuclei produced by meiosis contains one or the other member of the pair found in the diploid mother cell, but never both.

Selective permeability • A characteristic of a membrane, allowing certain substances to pass through while other substances are excluded.

Selfish act • A behavioral act that benefits its performer but harms the recipients.

Semelparous organism • An organism that reproduces only once in its lifetime. (Contrast with iteroparous.)

Semen (see' men) [L.: seed] • The thick, whitish liquid produced by the male reproductive organ in mammals, containing the sperm.

Semicircular canals • Part of the vestibular system of mammals.

Semiconservative replication • The common way in which DNA is synthesized. Each of the two partner strands in a double helix acts as a template for a new partner strand. Hence, after replication, each double helix consists of one old and one new strand.

Seminiferous tubules • The tubules within the testes within which sperm production occurs.

Senescence [L. senescere: to grow old] • Aging; deteriorative changes with aging; the increased probability of dying with increasing age.

Sensory neuron • A neuron leading from a sensory cell to the central nervous system. (Contrast with motor neuron.)

Sepal (see' pul) • One of the outermost structures of the flower, usually protective in function and enclosing the rest of the flower in the bud stage.

Septum [L.: partition] • A membrane or wall between two cavities.

Sertoli cells • Cells in the seminiferous tubules that nurture the developing sperm.

Serum • That part of the blood plasma that remains after clots have formed and been removed.

Sessile (sess' ul) [L. sedere: to sit] • Permanently attached; not moving.

Set point • In a regulatory system, the threshold sensitivity to the feedback stimulus.

Sex chromosome • In organisms with a chromosomal mechanism of sex determination, one of the chromosomes involved in sex determination.

Sex linkage • The pattern of inheritance characteristic of genes located on the sex chromosomes of organisms having a chromosomal mechanism for sex determination.

Sexual selection • Selection by one sex of characteristics in individuals of the opposite sex. Also, the favoring of characteristics in one sex as a result of competition among individuals of that sex for mates.

Shoot • The aerial part of a vascular plant, consisting of the leaves, stem(s), and flowers.

Sieve tube • A column of specialized cells found in the phloem, specialized to conduct organic matter from sources (such as photo-synthesizing leaves) to sinks (such as roots). Found principally in flowering plants.

Sieve tube member • A single cell of a sieve tube, containing cytoplasm but relatively few organelles, with highly specialized perforated end walls leading to elements above and below.

Sign stimulus • The single stimulus, or one out of a very few stimuli, by which an animal distinguishes key objects, such as an enemy, or a mate, or a place to nest, etc.

Signal sequence • The sequence of a protein that directs the protein through a particular cellular membrane.

Signal transduction pathway • The series of biochemical steps whereby a stimulus to a cell (such as a hormone or neurotransmitter binding to a receptor) is translated into a response of the cell.

Signature principle • States that because of continuity, prebiotic processes should leave some trace in contemporary biochemistry. (Compare with principle of continuity.)

Silencer • A sequence of eukaryotic DNA that binds proteins that inhibit the transcription of an associated gene.

Silent mutations • Genetic changes that do not lead to a phenotypic change. At the molecular level, these are DNA sequence changes that, because of the redundancy of the genetic code, result in the same amino acids in the resulting protein. See synonymous mutation.

Similarity matrix • A matrix to compare the structures of two molecules constructed by adding the number of their amino acids that are identical or different

Sinoatrial node (sigh' no ay' tree al) • The pacemaker of the mammalian heart.

Sinus (sigh' nus) [L. sinus: a bend, hollow] • A cavity in a bone, a tissue space, or an enlargement in a blood vessel.

Skeletal muscle • See striated muscle.

Sliding filament theory • A proposed mechanism of muscle contraction based on formation and breaking of crossbridges between actin and myosin filaments, causing them to slide together.

Small intestine • The portion of the gut between the stomach and the colon, consisting of the duodenum, the jejunum, and the ileum.

Small nuclear ribonucleoprotein particle (snRNP) • A complex of an enzyme and a small nuclear RNA molecule, functioning in RNA splicing.

Smooth muscle • One of three types of muscle tissue. Usually consists of sheets of mononucleated cells innervated by the autonomic nervous system.

Society • A group of individuals belonging to the same species and organized in a cooperative manner; in the broadest sense, includes parents and their offspring.

Sodium-potassium pump • The complex protein in plasma membranes that is responsible for primary active transport; it pumps sodium ions out of the cell and potassium ions into the cell, both against their concentration gradients.

Solute • A substance that is dissolved in a liquid (solvent).

Solute potential • A property of any solution, resulting from its solute contents; it may be zero or have a negative value.

Solution • A liquid (solvent) and its dissolved solutes.

Solvent • A liquid that has dissolved or can dissolve one or more solutes.

Somatic [Gr. soma: body] • Pertaining to the body, or body cells (rather than to germ cells).

Somite (so' might) • One of the segments into which an embryo becomes divided longitudinally, leading to the eventual segmentation of the animal as illustrated by the spinal column, ribs, and associated muscles.

Spatial summation • In the production or inhibition of action potentials in a postsynaptic neuron, the interaction of depolarizations and hyperpolarizations produced by several terminal boutons.

GLOSSARY

Spawning • The direct release of sex cells into the water.

Speciation (spee' shee ay' shun) • The process of splitting one population into two populations that are reproductively isolated from one another.

Species (spee' shees) [L.: kind] • The basic lower unit of classification, consisting of a population or series of populations of closely related and similar organisms. The more narrowly defined "biological species" consists of individuals capable of interbreeding freely with each other but not with members of other species.

Species diversity • A weighted representation of the species of organisms living in a region; large and common species are given greater weight than are small and rare ones. (Contrast with species richness.)

Species richness • The number of species of organisms living in a region. (Contrast with species diversity)

Specific heat • The amount of energy that must be absorbed by a gram of a substance to raise its temperature by one degree centigrade. By convention, water is assigned a specific heat of one.

Sperm [Gr. sperma: seed] • A male reproductive cell.

Spermatocyte (spur mat' oh site) [Gr. sperma: seed + kytos: cell] • The cell that gives rise to the sperm in animals.

Spermatogenesis (spur mat' oh jen' e sis) [Gr. sperma: seed + genesis: source] • Male gametogenesis, leading to the production of sperm.

Spermatogonia • Undifferentiated germ cells that give rise to primary spermatocytes and hence to sperm.

Sphincter (sfing'k' ter) [Gr. sphinkter: that which binds tight] • A ring of muscle that can close an orifice, for example at the anus.

Spindle apparatus • An array of microtubules stretching from pole to pole of a dividing nucleus and playing a role in the movement of chromosomes at nuclear division. Named for its shape.

Spiracle (spy' rih kel) [L. spirare: to breathe] • An opening of the tracheal respiratory system of terrestrial arthropods.

Spiteful act • A behavioral act that harms both the actor and the recipient of the act.

Spliceosome • An RNA-protein complex that splices out introns from eukaryotic pre-mRNAs.

Splicing • The removal of introns and connecting of exons in eukaryotic pre-mRNAs.

Spontaneous generation • The idea that life is generated continually from nonliving matter. Usually distinguished from the current idea that life evolved from nonliving matter under primordial conditions at an early stage in the history of earth.

Spontaneous reaction • A chemical reaction which will proceed on its own, without any outside influence. A spontaneous reaction need not be rapid.

Sporangium (spor an' gee um) [Gr. spora: seed + angeion: vessel or reservoir] • In plants and fungi, any specialized structure within which one or more spores are formed.

Spore [Gr. spora: seed] • Any asexual reproductive cell capable of developing into an adult plant without gametic fusion. Haploid spores develop into gametophytes, diploid spores into sporophytes. In prokaryotes, a resistant cell capable of surviving unfavorable periods.

Sporophyte (spor' o fyte) [Gr. spora: seed + phyton: plant] • In plants with alternation of generations, the diploid phase that produces the spores. (Contrast with gameto-phyte.)

Stabilizing selection • Selection against the extreme phenotypes in a population, so that the intermediate types are favored. (Contrast with disruptive selection.)

Stamen (stay' men) [L.: thread] • A male (pollen-producing) unit of a flower, usually composed of an anther, which bears the pollen, and a filament, which is a stalk supporting the anther.

Starch [O.E. stearc: stiff] • An or-linked polymer of glucose; used by plants as a means of storing energy and carbon atoms.

Start codon • The mRNA triplet (AUG) that acts as signals for the beginning of translation at the ribosome. (Compare with stop codons. There are a few minor exceptions to these codons.)

Stasis • Period during which little or no evolutionary change takes place within a lineage or groups of lineages.

Statocyst (star' oh sist) [Gk. statos: stationary + kystos: pouch] • An organ of equilibrium in some invertebrates.

Statolith (stat' oh lith) [Gk. statos: stationary + lithos: stone] • A solid object that responds to gravity or movement and stimulates the mechanoreceptors of a statocyst.

Stele (steel) [Gr. stele: pillar] • The central cylinder of vascular tissue in a plant stem.

Stem cell • A cell capable of extensive proliferation, generating more stem cells and a large clone of differentiated progeny cells, as in the formation of red blood cells.

Step cline • A sudden change in one or more traits of a species along a geographical gradient.

Steroid • Any of numerous lipids based on a 17-carbon atom ring system.

Sticky ends • On a piece of two-stranded DNA, short, complementary, one-stranded regions produced by the action of a restriction endonuclease. Sticky ends allow the joining of segments of DNA from different sources.

Stigma [L.: mark, brand] • The part of the pistil at the apex of the style, which is receptive to pollen, and on which pollen germinates.

Stimulus • Something causing a response; something in the environment detected by a receptor.

Stolon • A horizontal stem that forms roots at intervals.

Stoma (plural: stomata) [Gr. stoma: mouth, opening] • Small opening in the plant epidermis that permits gas exchange; bounded

by a pair of guard cells whose osmotic status regulates the size of the opening.

Stop codons • Triplets (UAG, UGA, UAA) in mRNA that act as signals for the end of translation at the ribosome. (See also

start codon. There are a few minor exceptions to these codons.)

Stratosphere • The part of the atmosphere above the troposphere; extends upward to approximately 50 kilometers above the surface of the earth; contains very little water.

Stratum (plural strata) • A layer or sedimentary rock laid down at a particular time in a past.

Striated muscle • Contractile tissue characterized by multinucleated cells containing highly ordered arrangements of actin and myosin microfilaments. Also known as skeletal muscle.

Stroma • The fluid contents of an organelle, such as a chloroplast.

Stromatolite • A composite, flat-to-domed structure composed of successive mineral layers. Some are known to be produced by the action of bacteria in salt or fresh water, and some ancient ones are considered to be evidence for early life on the earth.

Structural formula • A representation of the positions of atoms and bonds in a molecule.

Structural gene • A gene that encodes the primary structure of a protein.

Style [Gr. stylos: pillar or column] • In flowering plants, a column of tissue extending from the tip of the ovary, and bearing the stigma or receptive surface for pollen at its apex.

Sub- [L.: under] • A prefix often used to designate a structure that lies beneath another or is less than another. For example, subcutaneous, subspecies.

Submucosa • (sub mew koe' sah) • The tissue layer just under the epithelial lining of the lumen of the digestive tract. (Contrast with mucosa.)

Substrate (sub' strayte) • (1) The molecule or molecules on which an enzyme exerts catalytic action. (2) The base material on which an organism lives.

Substrate level phosphorylation • ATP formation resulting from direct transfer of a phosphate group to ADP from an intermediate in glycolysis. (Contrast with oxidative phosphorylation.)

Succession • In ecology, the gradual, sequential series of changes in species composition of a community following a disturbance.

Sulcus (plural: sulci) [L. sulcare: to plow] • The valleys or creases between the raised portions of the convoluted surface of the brain. (Contrast to gyrus.)

Sulfhydryl group • The —SH group.

Summation • The ability of a neuron to fire action potentials in response to numerous subthreshold postsynaptic potentials arriving simultaneously at differentiated places on the cell, or arriving at the same site in rapid succession.

SSAR\

Surface area-to-volume ratio • I or an) cell, organism, or geometrical solid, the ratio of surface area to volume; this is an important factor in setting an upper limit on the size a cell or organism can attain.

Surfactant • \ substance that decreases the

surface tension of a liquid. Lung surfactant secreted by cells of the alveoli, is mostly phospholipid and decreases the amount of work necessary to inflate the lungs.

Symbiosis (sim' bee oh' sis) [C.r.: to live together] • The living together of two or more species in a prolonged and intimate ecological relationship. (See parasitism, commensalism, mutualism.)

Symmetry • In biology, the property that two halves of an object are mirror images of each other. (See bilateral symmetry and biradial symmetry.)

Sympathetic nervous system • A division of the autonomic (involuntary) nervous system. Its activities include increasing blood pressure and acceleration of the heartbeat. The neurotransmitter at the sympathetic terminals is epinephrine or norepinephrine. (Contrast with parasympathetic nervous system.)

Sympatric speciation (sim pat' rik) [Gr. si/w: same + patria: homeland] • The occurrence of genetic reproduction isolation and the subsequent formation of new species without any physical separation of the subpopulation. (Contrast with allopatric speciation, parapatric speciation.)

Symplast • The continuous meshwork of the interiors of living cells in the plant body, resulting from the presence of plasmodesmata. (Contrast with apoplast.)

Symport • A membrane transport process that carries two substances in the same direction across the membrane. (Contrast with antiport.)

Synapse (sin' aps) [Gr. syn: together + liap-tcin: to fasten] • The narrow gap between the terminal bouton of one neuron and the dendrite or cell body of another.

Synapsis (sin ap' sis) • The highly specific parallel alignment (pairing) of homologous chromosomes during the first division of meiosis.

Synaptic vesicle • A membrane-bounded vesicle, containing neurotransmitter, which is produced in and discharged by the presynaptic neuron.

Syngamy (sing' guh mee) [Gr. sun-: together + gamos: marriage] • Union of gametes. Also known as fertilization.

Synonymous mutation • A mutation that substitutes one nucleotide for another but does not change the amino acid specified (i.e., UUA → UUG, both specifying leucine). (Compare with frame-shift mutation, mis-sense mutation, nonsense mutation.)

Synonymous substitution • The situation when a synonymous mutation becomes widespread in a population. Typically not influenced by natural selection, these substitutions can accumulate in a population. (Contrast with nonsynonymous substitution.)

Systematics • The scientific study of the diversity of organisms.

Systemic circulation • The part of the circulatory system serving those parts of the body other than the lungs or gills.

Systemin • The only polypeptide plant hormone; participates in response to tissue damage.

Systole (sis' tuh lee) [Gr.: contraction] • Contraction of a chamber of the heart, driving blood forward in the circulatory system.

T cell • A type of lymphocyte, involved in the cellular immune response. The final stages of its development occur in the thymus gland. (Contrast with B cell; see also cytotoxic T cell, helper T cell, suppressor T cell.)

T cell receptor • A protein on the surface of a T cell that recognizes the antigenic determinant for which the cell is specific.

T tubules • A system of tubules that runs throughout the cytoplasm of muscle fibers, through which action potentials spread.

Target cell • A cell with the appropriate receptors to bind and respond to a particular hormone or other chemical mediator.

Taste bud • A structure in the epithelium of the tongue that includes a cluster of chemoreceptors innervated by sensory neurons.

TATA box • An eight-base-pair sequence, found about 25 base pairs before the starting point for transcription in many eukaryotic promoters, that binds a transcription factor and thus helps initiate transcription.

Taxis (tak' sis) [Gr. taxis: arrange, put in order] • The movement of an organism in a particular direction with reference to a stimulus. A taxis usually involves the employment of one sense and a movement directly toward or away from the stimulus, or else the maintenance of a constant angle to it. Thus a positive phototaxis is movement toward a light source, negative geotaxis is movement upward (away from gravity), and so on.

Taxon • A unit in a taxonomic system.

Taxonomy (taks on' oh me) [Gr. taxis: arrange, classify] • The science of classification of organisms.

Telomeres (tee' lo merz) [Gr. telos: end] • Repeated DNA sequences at the ends of eukaryotic chromosomes.

Telophase (tee' lo phase) [Gr. telos: end] • The final phase of mitosis or meiosis during which chromosomes became diffuse, nuclear envelopes reform, and nucleoli begin to reappear in the daughter nuclei.

Template • In biochemistry, a molecule or surface upon which another molecule is synthesized in complementary fashion, as in the replication of DNA. In the brain, a pattern that responds to a normal input but not to incorrect inputs.

Template strand • In a stretch of double-stranded DNA, the strand that is transcribed.

Temporal summation • In the production or inhibition of action potentials in a postsynaptic neuron, the interaction of depolarizations or hyperpolarizations produced by rapidly repeated stimulation of a single point.

Tendon • A collagen-containing band of tissue that connects a muscle with a bone.

Terrestrial (ter res' tree al) [L. terra: earth] • Pertaining to the land. (Contrast with aquatic, marine.)

Territory • A fixed area from which an animal or group of animals excludes other members of the same species by aggressive behavior or display.

Tertiary structure • In reference to a protein, the relative locations in three-dimensional space of all the atoms in the molecule. The overall shape of a protein. (Contrast with primary, secondary, and quaternary structures.)

Test cross • A cross of a dominant-phenotype individual (which may be either heterozygous or homozygous) with a homozy-

gous-recessive individual.

Testis (tes' tis) (plural: testes) [L.: witness] • The male gonad; that is, the organ that produces the male sex cells.

Testosterone (tes toss' tuhr own) • A male sex steroid hormone.

Tetanus [Gr. tetanos: stretched] • (1) In physiology, a state of sustained, maximal muscular contraction caused by rapidly repeated stimulation. (2) In medicine, an often-fatal disease ("lockjaw") caused by the bacterium *Clostridium tetani*.

Thalamus • A region of the vertebrate fore-brain; involved in integration of sensory input.

Thallus (thai' us) [Gr.: sprout] • Any algal body which is not differentiated into root, stem, and leaf.

Theory • An explanation or hypothesis that is supported by a wide body of evidence. (Contrast with hypothesis, paradigm.)

Thermoneutral zone • The range of temperatures over which an endotherm does not have to expend extra energy to thermoregulate.

Thermoreceptor • A cell or structure that responds to changes in temperature.

Thoracic cavity • The portion of the mammalian body cavity bounded by the ribs, shoulders, and diaphragm. Contains the heart and the lungs.

Thorax • In an insect, the middle region of the body, between the head and abdomen. In mammals, the part of the body between the neck and the diaphragm.

Thrombin • An enzyme that converts fibrinogen to fibrin, thus triggering the formation of blood clots.

Thrombus (throm' bus) [Gk. thrombos: clot] • A blood clot that forms within a blood vessel and remains attached to the wall of the vessel. (Contrast with embolus.)

GLOSSARY

Thylakoid • A flattened sac within a chloroplast. The membranes of the numerous thylakoids contain all of the chlorophyll in a plant, in addition to the electron carriers of photophosphorylation. Thylakoids stack to form grana.

Thymine • A nitrogen-containing base found in DNA.

Thymus • A ductless, glandular portion of the lymphoid system, involved in development of the immune system of vertebrates.

Thyroid [Gr. thyreos: door-shaped] • A two-lobed gland in vertebrates. Produces the hormone thyroxine.

Thyrotropic hormone • A hormone that is produced in the pituitary gland of amphibia such as frogs and transported in the bloodstream to the thyroid gland, inducing the thyroid gland to produce the thyroid hormone that regulates metamorphosis from tadpole to adult frog.

Tight junction • A junction between epithelial cells, in which there is no gap whatever between the adjacent cells. Materials may get through a tight junction only by entering the epithelial cells themselves.

Tissue • A group of similar cells organized into a functional unit and usually integrated with other tissues to form part of an organ such as a heart or leaf.

Tonus • A low level of muscular tension that is maintained even when the body is at rest.

Totipotency • In a cell, the condition of possessing all the genetic information and other capacities necessary to form an entire individual.

Toxigenicity [L. toxicum: poison] • The ability of a bacterium to produce chemical substances injurious to the tissues of the host organism.

Trachea (tray' kee ah) [Gr. trakhoia: a small tube] • A tube that carries air to the bronchi of the lungs of vertebrates, or to the cells of arthropods.

Tracheid (tray' kee id) • A distinctive conducting and supporting cell found in the xylem of nearly all vascular plants, characterized by tapering ends and walls that are pitted but not perforated.

Tracheophytes [Gr. trakhoia: a small tube + phyton: plant] • Those plants with xylem and phloem, including psilophytes, club mosses, horsetails, ferns, gymnosperms, and angiosperms. (Contrast with nontracheophytes.)

Trait • One form of a character: Eye color is a character; brown eyes and blue eyes are traits.

Transcription • The synthesis of RNA, using one strand of DNA as the template.

Transcription factors • Proteins that assemble on a eukaryotic chromosome, allowing RNA polymerase II to perform

transcription.

Transduction • (1) Transfer of genes from one bacterium to another, with a bacterial virus acting as the carrier of the genes. (2) In sensory cells, the transformation of a

stimulus (e.g., light energy, sound pressure waves, chemical or electrical stimulants) into action potentials.

Transfection • Uptake, incorporation, and expression of recombinant DNA.

Transfer cell • A modified parenchyma cell that transports solutes from its cytoplasm into its cell wall, thus moving the solutes from the symplast into the apoplast.

Transfer RNA (tRNA) • A category of relatively small RNA molecules (about 75 nucleotides). Each kind of transfer RNA is able to accept a particular activated amino acid from its specific activating enzyme, after which the amino acid is added to a growing polypeptide chain.

Transformation • Mechanism for transfer of genetic information in bacteria in which pure DNA extracted from bacteria of one genotype is taken in through the cell surface of bacteria of a different genotype and incorporated into the chromosome of the recipient cell.

Transgenic • Containing recombinant DNA incorporated into its genetic material.

Translation • The synthesis of a protein (polypeptide). This occurs on ribosomes, using the information encoded in messenger RNA.

Translocation • (1) In genetics, a rare mutational event that moves a portion of a chromosome to a new location, generally on a nonhomologous chromosome. (2) In vascular plants, movement of solutes in the phloem.

Transpiration [L. spirare . to breathe] • The evaporation of water from plant leaves and stem, driven by heat from the sun, and providing the motive force to raise water (plus ions) from the roots.

Transposable element • A segment of DNA that can move to, or give rise to copies at, another locus on the same or a different chromosome.

Triglyceride • A simple lipid in which three fatty acids are combined with one molecule of glycerol.

Triplet • See codon.

Triplet repeat • Occurrence of repeated triplet of bases in a gene, often leading to genetic disease, as does excessive repetition of CGG in the gene responsible for fragile-X syndrome.

Triploblastic • Having three cell layers. (Contrast with diploblastic.)

Trisomic • Containing three, rather than two members of a chromosome pair.

tRNA • See transfer RNA.

Trochophore (troke' o fore) [Gr. troches: wheel + phoreus: bearer] • The free-swimming larva of some annelids and mollusks, distinguished by a wheel-like band of cilia around the middle, and indicating an evolutionary relationship between these two groups.

Trophic level • A group of organisms united by obtaining their energy from the same part of the food web of a biological community.

Tropic hormones • Hormones of the anterior pituitary that control the secretion of hormones by other endocrine glands.

Tropism [Gr. tropos: to turn] • In plants, growth toward or away from a stimulus such as light (phototropism) or gravity (gravitropism).

Tropomyosin (troe poe my' oh sin) • A protein that, along with actin, constitutes the thin filaments of myofibrils. It controls the interactions of actin and myosin necessary for muscle contraction.

Troposphere • The atmospheric zone reaching upward approximately 17 km in the tropics and subtropics but only to about 10 km at higher latitudes. The zone in which virtually all the water vapor in the atmosphere is located.

Trypsin • A protein-digesting enzyme. Secreted by the pancreas in its inactive form (trypsinogen), it becomes active in the duodenum of the small intestine.

T-tubules • A set of transverse tubes that penetrates skeletal muscle fibers and terminates in the sarcoplasmic reticulum. The T-system transmits impulses to the sacs, which then release Ca²⁺ to initiate muscle contraction.

Tube nucleus • In a pollen tube, the haploid nucleus that does not participate in double fertilization. (Contrast with generative nucleus.)

Tubulin • A protein that polymerizes to form microtubules.

Tumor • A disorganized mass of cells, often growing out of control. Malignant tumors spread to other parts of the body.

Tumor suppressor genes • Genes which, when homozygous mutant, result in cancer. Such genes code for protein products that inhibit cell proliferation.

Twitch • A single unit of muscle contraction.

Tympanic membrane [Gr. tympanum: drum] • The eardrum.

Umbilical cord • Tissue made up of embryonic membranes and blood vessels that connects the embryo to the placenta in eutherian mammals.

Understory • The aggregate of smaller plants growing beneath the canopy of dominant plants in a forest.

Unicellular (yoon' e sell' yer ler) [L. unus: one + cella: chamber] • Consisting of a single cell; as for example a unicellular organism. (Contrast with multicellular.)

Uniport • A membrane transport process that carries a single substance. (Contrast with antiport, symport.)

Unsaturated hydrocarbon • A compound containing only carbon and hydrogen atoms. One or more pairs of carbon atoms are connected by double bonds.

Upwelling • The upward movement of nutrient-rich, cooler water from deeper layers of the ocean.

SAIN

Urea • A compound serving as the main excreted form of nitrogen by many animals, including mammals.

Ureotelic • Describes an organism in which the final product of the breakdown of nitrogen-containing compounds (primarily proteins) is urea. (Contrast with ammonotelic, uricotelic.)

Ureter (your* uh tur) [Gr. ouron: urine] • A long duct leading from the vertebrate kidney to the urinary bladder or the cloaca.

Urethra (you ree' thra) [Gr. ouron: urine] • In most mammals, the canal through which urine is discharged from the bladder and which serves as the genital duct in males.

Uric acid • A compound that serves as the main excreted form of nitrogen in some animals, particularly those which must conserve water, such as birds, insects, and reptiles.

Uricotelic • Describes an organism in which the final product of the breakdown of nitrogen-containing compounds (primarily proteins) is uric acid. (Contrast with ammonotelic, ureotelic.)

Urinary bladder • A structure that receives urine from the kidneys via the ureter, stores it, and expels it periodically through the urethra.

Urine (you' rin) [Gk. ouron: urine] • In vertebrates, the fluid waste product containing the toxic nitrogenous by-products of protein and amino acid metabolism.

Uterus (yoo' ter us) [L.: womb] • The uterus or womb is a specialized portion of the female reproductive tract in certain mammals. It receives the fertilized egg and nurtures the embryo in its early development.

Vaccination • Injection of virus or bacteria or their proteins into the body, to induce immunization. The injected material is usually attenuated (weakened) before injection.

Vacuole (vac' yew ole) [Fr.: small vacuum] • A liquid-filled cavity in a cell, enclosed within a single membrane. Vacuoles play a wide variety of roles in cellular metabolism, some being digestive chambers, some storage chambers, some waste bins, and so forth.

Vagina (vuh jine' uh) [L.: sheath] • In female mammals, the passage leading from the external genital orifice to the uterus; receives the copulatory organ of the male in mating.

van der Waals interaction • A weak attraction between atoms resulting from the interaction of the electrons of one atom with the nucleus of the other atom. This attraction is about one-fourth as strong as a hydrogen bond.

Variable regions • The part of an immunoglobulin molecule or T-cell receptor that includes the antigen-binding site.

Vascular (vas' kew lar) • Pertaining to organs and tissues that conduct fluid, such as blood vessels in animals and phloem and xylem in plants.

Vascular bundle • In vascular plants, a strand of vascular tissue, including conducting cells of xylem and phloem as well as thick-walled fibers.

Vascular ray • In vascular plants, radially oriented sheets of cells produced by the vascular cambium, carrying materials

laterally between the wood and the phloem.

Vascular tissue system • The conductive system of the plant, consisting primarily of xylem and phloem. (Contrast with dermal tissue system, ground tissue system.)

Vasopressin • See antidiuretic hormone.

Vector • (1) An agent, such as an insect, that carries a pathogen affecting another species. (2) A plasmid or virus that carries an inserted piece of DNA into a bacterium for cloning purposes in recombinant DNA technology.

Vegetal hemisphere • The lower portion of some animal eggs, zygotes, and embryos, in which the dense nutrient yolk settles. The vegetal pole refers to the very bottom of the egg or embryo. (Contrast with animal hemisphere.)

Vegetative • Nonreproductive, or nonflowering, or asexual.

Vein [L. vena: channel] • A blood vessel that returns blood to the heart. (Contrast with artery.)

Ventral [L. venter, belly, womb] • Toward or pertaining to the belly or lower side. (Contrast with dorsal.)

Ventricle • A muscular heart chamber that pumps blood through the body.

Vernalization [L. vernalis: belonging to spring] • Events occurring during a required chilling period, leading eventually to flowering.

Vertebral column • The jointed, dorsal column that is the primary support structure of vertebrates.

Vertebrate • An animal whose nerve cord is enclosed in a backbone of bony segments, called vertebrae. The principal groups of vertebrate animals are the fishes, amphibians, reptiles, birds, and mammals.

Vessel [L. vasculum: a small vessel] • In botany, a tube-shaped portion of the xylem consisting of hollow cells (vessel elements) placed end to end and connected by perforations. Together with tracheids, vessel elements conduct water and minerals in the plant.

Vestibular apparatus (ves tib' yew lar) [L. vestibulum: an enclosed passage] • Structures associated with the vertebrate ear; these structures sense changes in position or momentum of the head, affecting balance and motor skills.

Vestigial (ves tij' ee al) [L. vestigium: footprint, track] • The remains of body structures that are no longer of adaptive value to the organism and therefore are not maintained by selection.

Vicariance (vye care' ee unce) [L. vicus: change] • The splitting of the range of a taxon by the imposition of some barrier to dispersal; its members.

Vicariant distribution • A distribution resulting from the disruption of a formerly continuous range by a vicariant event.

Villus (viT lus) (plural: villi) [L.: shaggy hair] • A hairlike projection from a membrane; for example, from many gut walls.

Virion (veer' e on) • The virus particle, the minimum unit capable of infecting a cell.

Viroid (vye' roid) • An infectious agent consisting of a single-stranded RNA molecule with no protein coat; produces diseases in plants.

Virus [L.: poison, slimy liquid] • Any of a group of ultramicroscopic infectious particles constructed of nucleic acid and protein (and, sometimes, lipid) that can reproduce only in living cells.

Visceral mass • The major internal organs of a mollusk.

Vitamin [L. vita: life] • Any one of several structurally unrelated organic compounds that an organism cannot synthesize itself, but nevertheless requires in small quantity for normal growth and metabolism.

Viviparous (vye vip' uh rus) [L. vivus: alive] • Reproduction in which fertilization of the egg and development of the embryo occur inside the mother's body. (Contrast with oviparous.)

Waggle dance • The running movement of a working honey bee on the hive, during which the worker traces out a repeated figure eight. The dance contains elements that transmit to other bees the location of the food.

Water potential • In osmosis, the tendency for a system (a cell or solution) to take up water from pure water, through a differentially permeable membrane. Water flows toward the system with a more negative water potential. (Contrast with osmotic potential, turgor pressure.)

Water vascular system • The array of canals and tubelike appendages that serves as the circulatory system, locomotory system, and food-capturing system of many echinoderms; is in direct connection with the surrounding sea water.

Wavelength • The distance between successive peaks of a wave train, such as electromagnetic radiation.

Wild type • Geneticists' term for standard or reference type. Deviants from this standard, even if the deviants are found in

the wild, are said to be mutant.

Xanthophyll (zan' tho fill) [Gr. xanthos: yellowish-brown + phyllon: leaf] • A yellow or orange pigment commonly found as an accessory pigment in photosynthesis, but found elsewhere as well. An oxygen-containing carotenoid.

X-linked (also called sex-linked) • A character that is coded for by a gene on the X chromosome.

Xerophyte (zee' row fyte) [Gr. xerox: dry + pkyton: plant] • A plant adapted to an environment with a limited water supply.

Xylem (zy' lum) [Gr. xylon: wood] • In vascular plants, the woody tissue that conducts water and minerals; xylem consists, in various plants, of tracheids, vessel elements, fibers, and other highly specialized cells.

GLOSSARY

Yeast artificial chromosome • A laboratory-made DNA molecule containing sequences of yeast chromosomes (origin of replication, telomeres, centromere, and selectable markers) so that it can be used as a vector in yeast.

Yolk • The stored food material in animal eggs, usually rich in protein and lipid.

Z-DNA • A form of DNA in which the molecule spirals to the left rather than to the right.

Zooplankton (zoe' o plang ton) [Gr. zoon: animal + planktos: wandering] • The animal portion of the plankton.

Zoospore (zoe' o spore) [Gr. zoon: animal + spora: seed] • In algae and fungi, any swimming spore. May be diploid or haploid.

Zygote (zye' gote) [Gr. zygos: yoked] • The cell created by the union of two gametes, in which the gamete nuclei are also fused. The earliest stage of the diploid generation.

Zymogen • An inactive precursor of a digestive enzyme secreted into the lumen of the gut, where a protease cleaves it to form the active enzyme.

Authors' Photograph

Christopher Small

Table of Contents Photographs

Golgi: © Biophoto Associates/Science

Source/Photo Researchers, Inc. Fibroblast: © Dennis Kunkel, U. Hawaii. Plasmid: © A. B. Dowsett/Science Photo

Library/Photo Researchers, Inc. Protein: © James King-Holmes/OCMS/

Science Photo Library/Photo Researchers,

Inc. Fossils: © Tom and Therisa Stack/Tom Stack

and Assoc. Starfish: © Darrell Gulin/DRK PHOTO. Fireweed: © Stephen J. Krasemann/

DRK PHOTO. Blueberries: © Pat O'Hara/DRK PHOTO. , Fetus: © Science Pictures, Ltd. OSF/DRK

PHOTO. Polar bears: © Mike and Lisa Husar/

DRK PHOTO. Giraffes: © BIOS/Peter AAiold, Inc. Poppies: © Larry Ulrich/DRK PHOTO. Zebras: © Art Wolfe.

Part-Opener Photographs

Part 1: © K. R. Porter/Science Source/Photo

Researchers, Inc. Part 2: © Conly L. Rieder/BPS.* Part 3: © Tui de Roy/Minden Pictures. Part 4: © Dan Dempster/Dembinsky Photo

Assoc. Part 5: © Gerry Ellis/Minden Pictures. Part 6: © Michael Fogden/DRK PHOTO. Part 7: © Doug Perrine/DRK PHOTO.

Chapter 1 Opener: © Enric Marti /Associated Press. 1.3: © T. Stevens and P. McKin-ley/Photo Researchers, Inc. 1.4: © Dennis Kunkel, U. Hawaii. 1.5: © Science Pictures Limited/CORBIS. 1.6 larva: © Valorie Hodgson/Visuals Unlimited. 1.6 pupa: © Dick Poe/Visuals Unlimited. 1.6 butterfly: © Bill Beatty/Visuals Unlimited. 1.7: © K. and K. Amman/Planet Earth Pictures. 1.8n: © Staffan Widstrand. 1.8b: © Joe MacDonald/ Tom Stack & Assoc. 1.8c: © Steve Kaufman/ Peter Arnold, Inc. 1.8d: © Luiz C. Marigo/ Peter Arnold, Inc. 1.11: J. S. Bleakney, courtesy of J. S. Boates. 1.12: © T. Leeson/Photo Researchers, Inc. 1.13: © J. S. Boates.

*BPS = Biological Photo Service

Chapter 2 Opener: NASA. 2.3 left: © SIU/ Visuals Unlimited. 2.3 right: © SIU/Visuals Unlimited. 2.14: © Art Wolfe. 2.16: © P. Armstrong/Visuals Unlimited.

Chapter 3 Opener: © Dennis Kunkel, U. Hawaii. 3.6a,b,c; 3.7: © Dan Richardson. 3.14c left: © Biophoto Associates/Photo Researchers, Inc. 3.14c middle: © W. F. Schadel/ BPS. 3.14c right: © CNRI, Science World Enterprises/BPS. 3.15 upper: © Robert Brons/ BPS. 3.15 lower: © Peter J. Bryant/BPS. 3.18: © Dan Richardson.

Chapter 4 Opener: © Dennis Kunkel, U. Hawaii. 4.1: After N. Campbell, 1990. Biology, 2nd Ed., Benjamin Cummings Publishing Co. 4.3 upper row: © David M. Phillips/ Visuals Unlimited. 4.3 middle row, left: © Conly L. Rieder/BPS. 4.3 middle row, center: David Albertini, Tufts U. School of Medicine. 4.3 middle row, right: © M. Abbey/ Photo Researchers, Inc. 4.3 bottom row, left: © D. P. Evenson/BPS. 4.3 bottom row, center: © BPS. 4.3 bottom row, right: © L. Andrew Staehelin, U. Colorado. 4.4: © J. J. Cardamone Jr. & B. K. Pugashetti/BPS. 4.5: © Stanley C. Holt/BPS. 4.6a: © J. J. Cardamone Jr./BPS. 4.6c: S. Abraham & E. H. Beachey, VA Medical Center, Memphis, TN. 4.7 centrioles: © Barry F. King/BPS. 4.7 mitochondrion: © K. Porter, D. Fawcett/Visuals Unlimited. 4.7 rough ER: © Don Fawcett/Science Source/ Photo Researchers, Inc. 4.7 plasma membrane: J. David Robertson, Duke U. Medical Center. 4.7 peroxisome: © E. H. Newcomb & S. E. Frederick/BPS. 4.7 nucleolus: © Richard Rodewald/BPS. 4.7 golgi apparatus: © L. Andrew Staehelin, U. Colorado. 4.7 smooth ER: © Don Fawcett, D. Friend/Science Source/ Photo Researchers, Inc. 4.7 ribosome: From Boublik et al., 1990, The Ribosome, p. 177. Courtesy of American Society for Microbiology. 4.7 cell wall: © David M. Phillips/Visuals Unlimited. 4.7 chloroplast: © W. P. Wer-gin, courtesy of E. H. Newcomb/BPS. 4.8 upper: © Richard Rodewald/BPS. 4.8 lower: © Don W. Fawcett/Photo Researchers, Inc. 4.9a: © Barry King, U. California, Davis/ BPS. 4.9b: © Biophoto Associates/Science Source/Photo Researchers, Inc. 4.10: From Aebi, U., et al., 1986. Nature 323:560-564. © Macmillan Publishers Ltd. 4.11: © Don Fawcett/Visuals Unlimited. 4.12: © L. Andrew Staehelin, U. Colorado. 4.13: © K. G. Murti/

Visuals Unlimited. 4.14: © K. Porter, D. Fawcett/Visuals Unlimited. 4.15: © W. P. Wergin, courtesy of E. H. Newcomb/BPS. 4.16a: © John Durham/Science Photo Library/Photo Researchers, Inc. 4.16b: © Ed Reschke/Peter Arnold, Inc. 4.16c: © Chuck Davis/Tony Stone Images. 4.17a: © Richard Shiell/Animals Animals. 4.17a inset: © Richard Green/Photo Researchers, Inc. 4.17b: © G. Buttner/Naturbild/OKAPIA/ Photo Researchers, Inc. 4.17b inset: © R. R. Dute. 4.19: © E. H. Newcomb & S. E. Frederick/BPS. 4.20: © M. C. Ledbetter, Brook-haven National Laboratory. 4.21: Courtesy of Vic Small, Austrian Academy of Sciences, Salzburg. 4.22: © Nancy Kedersha/Im-munogen/Science Photo Library/Custom Medical Stock Photo. 4.23: © N. Hirokawa. 4.24: © W. L. Dentler/BPS. 4.25: B. J. Schnapp et al., 1985. Cell 40:455. Courtesy of B. J. Schnapp, R. D. Vale, M. P. Sheetz, and T. S. Reese. 4.26: © Barry F. King/BPS. 4.27: © David M. Phillips/Visuals Unlimited. 4.28 left: Courtesy of David Sadava. 4.28 upper right: © J. Gross, Biozentrum/Science Photo Library/Photo Researchers, Inc. 4.28 lower right: © From J. A. Buckwalter and L. Rosenberg, 1983. Coll. Rel. Res. 3:489. Courtesy of L. Rosenberg.

Chapter 5 Opener: © Reuters Newmedia Inc./CORBIS. 5.2: After L. Stryer, 1981. Biochemistry, 2nd Ed., W. H. Freeman. 5.3: © L. Andrew Staehelin, U. Colorado. 5.6a: © D. S. Friend, U. California, San Francisco. 5.6b: © Darcy E. Kelly, U. Washington. 5.6c: Courtesy of C. Peracchia. 5.15: Courtesy of J. Casley-Smith. 5.16: From M. M. Perry, 1979. / Cell Sci. 39:26.

Chapter 6 Opener: © Geoff Tompkinson/ Science Photo Library/Photo Researchers, Inc. 6.2: © Jonathan Scott/Masterfile. 6.7: © Jeff J. Daly/Visuals Unlimited. 6.15: © The Mona Group. 6.17: © Clive Freeman, The Royal Institution/Photo Researchers, Inc. 6.19: © The Mona Group.

Chapter 7 Opener: © Catherine Ursillo/ Photo Researchers, Inc. 7.13: © Ephraim Racker/BPS.

Chapter 8 Opener: © C. S. Lobban/BPS. 8.1: © C. G. Van Dyke/Visuals Unlimited. 8.15: © Lawrence Berkeley National Labora-

ton 8.18 8 20: ! E. H. Newcomb & S. E. Frederick BIN. S.21 left, C Arthur R. Hill/ Visuals Unlimited. S.21 right. © David Matherly ^Visuals Unlimited.

Chapter 9 Opener. S> Nancy Kedersha/Sci-ence Plioto I ibrarv Photo Researchers, Inc. 9.1a/r. o John D. Cunningham, Visuals, Unlimited. 9.1fc © David M. Phillips/Visuals Unlimited. 9.2: Ruth Kavenoff, Designer-genes Ltd., P.O. Box 100, Del Mar, CA 90214. [ohn 1. Cardamone Jr./BPS. 9.6: © G. F. Bahr/BPS. 9.7 upper inset: © A. L. Olins/ BPS. 9.7 lower inset: © Biophoto Associates/ Science Source/Photo Researchers, Inc. 9.8: © Andrew S. Bajer, U. Oregon. 9.9b: © Conly L. Rieder/BPS. 9.10(7: © T. E. Schroed-er/BPS. 9.10fr: © B. A. Palevitz, U. Wisconsin, courtesy of E. H. Newcomb/BPS. 9.11: © Gary T. Cole/BPS. 9.12a: © Andrew Svred/Science Photo Library/Photo Researchers, Inc. 9.12b: © E. Webber/Visuals Unlimited. 9.12c: © Bill Kamin/Visuals Unlimited. 9.13: © Dr. Thomas Ried and Dr. Evelin Schrock, NIH. 9.14: © C. A. Hasen-kampf/BPS. 9.15: © Klaus W. Wolf, U. West Indies. 9.19b: © Gopal Murti/Photo Researchers, Inc.

Chapter 10 Opener: © David H. Wells/ CORBIS. 10.2: © R. W. Van Norman /Visuals Unlimited. 10.12: Courtesy the American Netherland Dwarf RabbitClub. 10.15: © NCI/Photo Researchers, Inc. 10.16: Courtesy of Pioneer Hi-Bred International, Inc. 10.17: After N. Campbell, 1990. Biology, 2nd Ed., Benjamin Cummings Publishing Co. 10.26: © Science VU/Visuals Unlimited. Bay scallops: © Barbara J. Miller/BPS.

Chapter 11 Opener: © From coordinates provided by N. Geacintov, NYU. 11.2: © Lee D. Simon/Photo Researchers, Inc. 11.4: Courtesy of Prof. M. H. F. Wilkins, Dept. of Biophysics, King's College, U. London. 11.6a: © A. Barrington Brown/Photo Researchers, Inc. 11.6b: © Dan Richardson.

Chapter 12 Opener: © David Wrobel/Visu-als Unlimited. 12.7: © Dan Richardson. 12.13b: Courtesy of J. E. Edstrom and EMBO }. 12.17a: © Stanley Flegler/Visuals Unlimited. 12.17b: © Stanley Flegler/Visuals Unlimited.

Chapter 13 Opener: © Rosenfeld Images LTD/Photo Researchers, Inc. 13.1a: © Dennis Kunkel, U. Hawaii. 13.1b: © E.O.S./ Gelderblom/Photo Researchers, Inc. 13.1c: © Dennis Kunkel, U. Hawaii. 13.8: Courtesy of L. Caro and R. Curtiss. 13.21: Based on an illustration by Anthony R. Kerlavage, Institute for Genomic Research. Science 269: 449-604 (1995).

Chapter 14 Opener: © Andrew Syred / Tony Stone images. 14.8: © Tiemeier et al., 1978. Cell 14:237-246. 14.18: Courtesy

of Murray L. Barr, U. Western Ontario. 14.19: Courtesy of O. L. Miller, Jr.

Chapter 15 Opener: © Victoria Blackie/ Tony Stone Images. 15.3 inset: © Biophoto Associates/Photo Researchers, Inc. 15.4: © From de Vos et al., 1992. Science 255: 306-312. 15.14: © Stephen A. Strieker, courtesy of Molecular Probes, Inc.

Chapter 16 Opener: © Yorgos Nikas, Karolinska Institute. 16.4: © Roddy Field, the Roslin Institute. 16.5: Courtesy of T. Wakayama and R. Yanagimachi. 16.9: J. E. Sulston and H. R. Horvitz, 1977. Dev. Bio. 56:100. 16.10b; 16.12 left: Courtesy of J. Bowman. 16.12 right: Courtesy of Detlef Weigel. 16.13: Courtesy of W. Driever and C. Niisslein-Vollhard. 16.20: Courtesy of F. R. Turner, Indiana U.

Chapter 17 Opener: Courtesy of Nexia Biotechnologies, Inc. 17.2: © Philippe Plailly/Photo Researchers, Inc. 17.7: Pamela Silver and Jason A. Kahana, courtesy of Chroma Technology. 17.16 left: © Custom Medical Stock Photography. 17.16 right: Courtesy of Ingo Potrykus, Swiss Federal Institute of Technology 17.18: © Bettmann/ CORBIS.

Chapter 18 Opener: Willard Centerwall, from Lyman, F. L. (ed.), 1963. Phenylketonuria. Charles C. Thomas, Springfield, IL. 18.5: C. Harrison et al., 1983. / Med. Genet. 20:280. 18.10: Courtesy of Harvey Levy and Cecelia Walraven, New England Newborn Screening Program. 18.13: © P. P. H. De-bruyn and Yongock Cho, U. Chicago/BPS.

Chapter 19 Opener: © Francis G. Mayer/ CORBIS. 19.4: © Dennis Kunkel, U. Hawaii. 19.10: © Dr. Gopal Murti/Science Photo Library/Photo Researchers, Inc. 19.15: A. Liepins, Sloan-Kettering Research Inst. 19.17: David Phillips/Science Source/Photo Researchers, Inc.

Chapter 20 Opener: © Robert Fried/Tom Stack & Assoc. 20.1: © Richard Coomber/ Planet Earth Pictures. 20.5: © Francois Gohier/The National Audubon Society Collection/Photo Researchers, Inc. 20.6: © W. B. Saunders/BPS. 20.9 left: © Ken Lucas/BPS. 20.9 right: © Stanley M. Awramik/BPS. 20.10: © Chip Clark. 20.11: © Tom McHugh/ Field Museum, Chicago/Photo Researchers, Inc. 20.12: © Chase Studios, Cedar creek, MO. 20.14: Transparency no. 5800 (3), photo by D. Finnin, painting by Robert J. Barber. Courtesy the Library, American Museum of Natural History.

Chapter 21 Opener: © Toshiyuki Yoshi-no/Nature Production. 21.1: © Science Photo Library/Photo Researchers, Inc. 21.2: Levi, W. 1965. Encyclopedia of Pigeon Breeds. T. F. H. Publications, Jersey City, NJ. (a,b: photos by R. L. Kienlen, courtesy of Ralston Purina Company; c,d: photos by Stauber.). 21.9: © Frank S. Balthis. 21.11: © Lincoln Nutting/The National Audubon Society Collection/Photo Researchers, Inc. 21.13(7: ©

C. Allan Morgan/Peter Arnold, Inc. 21.16: © Based on drawings produced by the Net-Spinner Web Program by Peter Fuchs and Thiemo Krink. 21.17: After D. Futuyma, 1987. Evolutionary Biology, 2nd Ed., Sinauer Associates, Inc. 21.20: Courtesy P. Brakefield and S. Carroll, from Brakefield et al., Nature 372:458- ^61. © Macmillan Publishers Ltd. 21.21fl: © Marilyn Kazmers/Dembinsky Photo Assoc. 21.21b: © Randy Morse/Tom Stack and Assoc.

Chapter 22 Opener: © Patti Murray/Animals Animals. 22.1(7: © Gary Meszaros/ Dembinsky Photo Assoc. 22.1b: © Lior Rubin/Peter Arnold, Inc. 22.7a: © Virginia P. Weinland/Photo Researchers, Inc. 22.7b: © Jose Manuel Sanchez de Lorenzo Caceres. 22.8: © Reed/Williams/Animals Animals. 22.10 upper, lower: © Peter J. Bryant/BPS. 22.10 center: © Kenneth Y. Kaneshiro, U. Hawaii. 22.13 left: © Peter K. Zimin-sky/Visuals Unlimited. 22.13 center: © Elizabeth N. Orians. 22.13 right © Noble Proctor/The National Audubon Society Collection /Photo Researchers, Inc.

Chapter 23 Opener: © Gary Brettnacher/ Adventure Photo & Film. 23.3 left: © Adam Jones/Dembinsky Photo Assoc. 23.3 right: © Brian Parker/Tom Stack & Assoc. 23.10(7: © Michael Giannechini/Photo Researchers, Inc. 23.10b: © Helen Carr/BPS. 23.10c: © Skip Moody/Dembinsky Photo Assoc.

Chapter 24 Opener: © John Reader/Science Photo Library/Photo Researchers, Inc. 24.2, 24.5: © Richard Alexander, U. Pennsylvania. 24.8: Courtesy of E. B. Lewis.

Chapter 25 Opener: © Mehau Kulyk/Sci-ence Photo Library/Photo Researchers, Inc. 25.1: © Stanley M. Awramik/BPS. 25.2: © Roger Ressmeyer/CORBIS. 25.5a: © Tom & Therisa Stack/Tom Stack & Assoc. 25.5b: © Gary Bell/Planet Earth Pictures.

Chapter 26 Opener: Photo by Ferran Garcia Pichel, from the cover of Science 284 (no. 5413). 26.1: © Kari Lounatmaa/Photo Researchers, Inc. 26.3(7: © David Phillips/ Photo Researchers, Inc. 26.3b: © R. Kessel-G. Shih/Visuals Unlimited. 26.3c: © Stanley Flegler/Visuals Unlimited. 26.4: © T. J. Beveridge/BPS. 26.5a: ©J. A. Breznak and H. S. Pankratz/BPS. 26.5b: © J. Robert Waaland/ BPS. 26.6: © George Musil/Visuals Unlimited. 26.7(7 left: © S. C. Holt/BPS. 26.7(7 center: © David M. Phillips/Visuals Unlimited. 26.7b left: © Leon J. LeBeau/BPS. 26.7b center: © A. J. J. Cardamone, Jr./BPS. 26.8: © Alfred Pasioka/Photo Researchers, Inc. 26.9: © Wolfgang Baumeister/Science Photo Library/Photo Researchers, Inc. 26.13: © Phil Gates, U. Durham/BPS. 26.14: © S. C. Holt/BPS. 26.15(7: © Paul W. Johnson/BPS. 26.15b: © H. S. Pankratz/BPS. 26.15c: © Bill Kamin/Visuals Unlimited. 26.16: © Science VU/Visuals Unlimited. 26.17: © Randall C. Cutlip/BPS. 26.18: © T. J. Beveridge/BPS.

26.19: © G. W. Willis/BPS. 26.20: © Science VU/Visuals Unlimited. 26.21: © Michael Gabridge/Visuals Unlimited. 26.23: © Krafft/ Hoa-qui/Photo Researchers, Inc. 26.24: © Martin G. Miller/Visuals Unlimited.

Chapter 27 Opener. © Mike Abbey/Visuals Unlimited. 27.1a: © David Phillips/Visuals Unlimited. 27.1b: © J. Paulin/Visuals Unlimited. 27.1c: © Randy Morse/Tom Stack & Assoc. 27.7a: © Christian Gautier/ Jacana/Photo Researchers, Inc. 27.7b: © Cabisco/Visuals Unlimited. 27.7c: © Alex Rakosy/Dembinsky Photo Assoc. 27.8: © David M. Phillips/Visuals Unlimited. 27.11: © Oliver Meckes/Photo Researchers, Inc. 27.12: © Sanford Berry/Visuals Unlimited. 27.14a: © Mike Abbey/Visuals

Unlimited. 27.14fr: © Dennis Kunkel, U. Hawaii. 27.14c,d: © Paul W. Johnson/BPS. 27.15b: © M. A. Jakus, NIH. 27.18(7: © Manfred Kage/Peter Arnold, Inc. 27.18b: © Biophoto Associates/Photo Researchers, Inc. 27.20a: © Joyce Photographies/The National Audubon Society Collection/Photo Researchers, Inc. 27.20b: © J. Robert Waaland/BPS. 27.21a: © Jeff Foott/Tom Stack & Assoc. 27.21b: © J. N. A. Lott/BPS. 27.23: © James W. Richardson/Visuals Unlimited. 27.24a: © Maria Scheffer/BPS. 27.24b: © J. N. A. Lott/BPS. 27.25a: © Cabisco/Visuals Unlimited. 27.25b: © Andrew J. Martinez/Photo Researchers, Inc. 27.25c: © Alex Rakosy/Dembinsky Photo Assoc. 27.31a: © Robert Brons/ BPS. 27.31b: © A. M. Siegelman/Visuals Unlimited. 27.32a: © Barbara J. Miller/BPS. 27.32b: © Cabisco/Visuals Unlimited. 27.33a: © D. W. Francis, U. Delaware. 27.33b: © David Scharf/Peter Arnold, Inc.

Chapter 28 Opener: © Fred Bruemmer/ DRK PHOTO. 28.1a: © Ron Dengler/Visuals Unlimited. 28.1b: © Larry Mellichamp/Visuals Unlimited. 28.4a,b: © J. Robert Waaland/BPS. 28.5a: © Rod Planck/Dembinsky Photo Assoc. 28.5b: © William Harlow/ Photo Researchers, Inc. 28.5c: © Science VU/Visuals Unlimited. 28.6: © Dr. David Webb, U. Hawaii. 28.7a: © Brian Enting/ Photo Researchers, Inc. 28.7b: © J. H. Troughton. 28.9: Figure information provided by Hermann Pfefferkorn, Dept. of Geology, U. Pennsylvania. Original oil painting by John Woolsey. 28.14a: © Ed Reschke/ Peter Arnold, Inc. 28.14b: © Cabisco/Visuals Unlimited. 28.15a: © J. N. A. Lott/BPS. 28.15b: © David Sieren/Visuals Unlimited. 28.16: © W. Ormerod/Visuals Unlimited. 28.17a: © Rod Planck/Dembinsky Photo Assoc. 28.17b: © Nuridsany et Perennou/ Photo Researchers, Inc. 28.17c: © Dick Keen/ Visuals Unlimited. 28.18: © L. West/Photo Researchers, Inc.

Chapter 29 Opener. © Marty Cordano/DRK PHOTO. 29.3: © Phil Gates/ BPS. 29.4a: © Roland Seitre/Peter Arnold, Inc. 29.4b: © Bernd Wittich/Visuals Unlimited. 29.4c: © M. Graybill/J. Hodder/BPS. 29.id: © Louisa Preston/Photo Researchers, Inc. 29.7a: © Dick Poe/Visuals Unlimited.

29.7b: © Richard Shiell. 29.7c: © Richard Shiell/Dembinsky Photo Assoc. 29.8a: © Richard Shiell. 29.8b: © Noboru Komine/Photo Researchers, Inc. 29.11a: © Inga Spence/Tom Stack & Assoc. 29.11b: © Holt Studios/Photo Researchers, Inc. 29.11c: © Catherine M. Pringle/BPS. 29.11d: © Inga Spence/Tom Stack & Assoc. 29.12: © U. California, Santa Cruz, and UCSC Arboretum. 29.12 inset: © Sandra K. Floyd, U. Colorado. 29.14a: © Ken Lucas/Visuals Unlimited. 29.14b: © Ed Reschke/Peter Arnold, Inc. 29.14c: © Adam Jones/Dembinsky Photo Assoc. 29.15a: © Richard Shiell. 29.15b: © Adam Jones/Dembinsky Photo Assoc. 29.15c: © Alan & Linda Detrick/The National Audubon Society Collection/Photo Researchers, Inc.

Chapter 30 Opener: © S. Nielsen/DRK PHOTO. 30.1a: © Inga Spence/Tom Stack & Assoc. 30.1b: © L. E. Gilbert/BPS. 30.1c: © G. L. Barron/BPS. 30.2: © David M. Phillips/ Visuals Unlimited. 30.4: © G. T. Cole/BPS. 30.5: © N. Allin and G. L. Barron/BPS. 30.7: © J. Robert Waaland/BPS. 30.8: © Gary R. Robinson /Visuals Unlimited. 30.9: © Tom Stack/Tom Stack & Assoc. 30.10: © John D. Cunningham/Visuals Unlimited. 30.11a: © Richard Shiell/Dembinsky Photo Assoc. 30.11b: © Matt Meadows/Peter Arnold, Inc. 30.12: © Andrew Syred/Science Photo Library/Photo Researchers, Inc. 30.14a: © Angelina Lax/Photo Researchers, Inc. 30.14b: © Manfred Danegger/Photo Researchers, Inc. 30.14c: © Stan Flegler/Visuals Unlimited. 30.15 inset: © Biophoto Associates/ Photo Researchers, Inc. 30.16a: © R. L. Peter-son/BPS. 30.16b: © Merton F. Brown/Visuals Unlimited. 30.17a: © Ed Reschke/Peter Arnold, Inc. 30.17b: © Gary Meszaros/Dem-binsky Photo Assoc. 30.18a: © J. N. A. Lott/ BPS.

Chapter 31 Opener: © Paolo Curto/The Image Bank. 31.5a: © Don Fawcett/Visuals Unlimited. 31.5b: © Christian Petron/Planet Earth Pictures. 31.5c: © Gillian Lythgoe/ Planet Earth Pictures. 31.6a: © Robert Brons/ BPS. 31.6b: © Tom & Therisa Stack/Tom Stack & Assoc. 31.6c: © Randy Morse/Tom Stack & Assoc. 31.7, 31.8, 31.9, 31.10: Adapted from Bayerand, F. M., and H. B. Owre, 1968. The Free-Living Lower Invertebrates, Macmillan Publishing Co. 31.11a: © G. Carleton Ray/Photo Researchers, Inc. 31.11b: © Fred Bavendam/Minden Pictures. 31.12: © David J. Wrobel/BPS. 31.13: From M. W. Martin, 2000. Science 288:841-845. 31.15a: © Fred McConnaughey/Photo Researchers, Inc. 31.17b: © James Solliday/BPS. 31.20a: © Chamberlain, MC/DRK PHOTO. 31.21: © David J. Wrobel/BPS. 31.22: © Jeff Mon-dragon. 31.24a: © Brian Parker/Tom Stack & Assoc. 31.24b: © Roger K. Burnard/BPS. 31.24c: © Stanley Breeden/DRK PHOTO. 31.24d: © R. R. Hessler, Scripps Institute of Oceanography. 31.26a: © Ken Lucas/Planet Earth Pictures. 31.26b: © Dave Fleetham/Tom Stack & Assoc. 31.26c: © Mike Sev-erns/Tom Stack & Assoc. 31.26a": © Milton

Rand/Tom Stack & Assoc. 31.26c: © Dave Fleetham/Tom Stack & Assoc. 31.26/: © A. Kerstitch/Visuals Unlimited.

Chapter 32 Opener: © John Mitchell/The National Audubon Society Collection/ Photo Researchers, Inc. 32.2: © Dr. Rick Hochberg, U. New Hampshire. 32.4: © R. Calentine/Visuals Unlimited. 32.5b,c: © James Solliday/BPS. 32.7a: © Doug Wech-sler. 32.7b: © Diane R. Nelson/Visuals Unlimited. 32.8: © Ken Lucas/Visuals Unlimited. 32.9a: © Joel Simon. 32.9b: © Fred Bruemmer/DRK PHOTO. 32.10a: © Peter J. Bryant/BPS. 32.10b: © David Maitland/ Masterfile. 32.10c: © W. M. Beatty/Visuals Unlimited. 32.10rf: © Robert Brons/BPS. 32.11a: © Henry W. Robison/Visuals Unlimited. 32.11b: © Stephen P. Hopkin/Planet Earth Pictures. 32.11c: © Peter David/Planet Earth Pictures. 32.lid: © A. Flowers & L. Newman/The National Audubon Society Collection/Photo Researchers, Inc. 32.13a: © Charles R. Wytttenbach/BPS. 32.13b: © William Leonard/DRK PHOTO. 32.15a: © David P. Maitland /Planet Earth Pictures. 32.15b: © Konrad Wothel/Minden Pictures. 32.15c: © Peter J. Bryant/BPS. 32.15d: © David Maitland /Masterfile. 32.15c: © Steve Nicholls/Planet Earth Pictures. 32.15/: © Brian Kenney/Planet Earth Pictures. 32.15g: © Simon D. Pollard/The National Audubon Society Collection/Photo Researchers, Inc. 32.15/;; © L. West/The National Audubon Society Collection/Photo Researchers, Inc.

Chapter 33 Opener: © Norbert Wu/DRK PHOTO. 33.3a: © Hal Beral/Visuals Unlimited. 33.3b: © Randy Morse/Tom Stack & Assoc. 33.3c: © Mark J. Thomas/Dembinsky Photo Assoc. 33.3d: © Randy Morse/Tom Stack & Assoc. 33.3c: © John A. Anderson/Animals Animals. 33.4: © C. R. Wytttenbach/BPS. 33.5: © Gary Bell/Masterfile. 33.6b, 33.9: © Norbert Wu/DRK PHOTO. 33.11a: © Dave Fleetham/Tom Stack & Assoc. 33.11b: © Marty Snyderman/Master-file. 33.12a: © Ken Lucas/Planet Earth Pictures. 33.12b: © Fred Bavendam/Minden Pictures. 33.12c: © Dave Fleetham/Visuals Unlimited. 33.12a 7 : © Dr. Paul A. Zahl/The National Audubon Society Collection/ Photo Researchers, Inc. 33.13: © Tom McHugh, Steinhart Aquarium/The National Audubon Society Collection/Photo Researchers, Inc. 33.15a: © Ken Lucas/BPS. 33.15b: © Nick Garbutt/Indri Images. 33.15c: © Art Wolfe. 33.19a: © Michael Fog-den/DRK PHOTO. 33.19b: © Joe McDonald/Tom

Stack & Assoc. 33.19c: © C. Alan Morgan/Peter Arnold, Inc. 33.19d: © Dave B. Fleetham/Tom Stack & Assoc. 33.19c: © Mark J. Thomas/Dembinsky Photo Assoc. 33.20a: Courtesy of Carnegie Museum of Natural History, Pittsburgh. 33.20b: Fossil from the Natural History Museum of Basel, photographed by Severino Dahint. 33.21a: © Joe McDonald/Tom Stack & Assoc. 33.21b: © John Shaw/Tom Stack & Assoc. 33.21c: © Skip Moody/Dembinsky

Photo Ed Kanze Dembin-

skj Photo \sstx Dave Watts/Tom

k & Vssoc. \323a ^ Art Wolfe. 33.23b: £ [anj Sauvanet Photo Researchers, Inc.

rians&Jud) Beste Animals Animals 3324a: 6 Rod Planck/Dembinsky Photo Assoc V3.2-] McDonald/Tom

Stack & Assoc I ; 24 © Doug Perrine/ Planet Earth Picture-]324d: © Erwin & PeggJ Bauer Iorn Stack & Assoc. 33.26a:© Art Wolfe. 33266: © Gary Milburn/Tom Stack & ^SSOC 33.27d: © Steve Kaufman/ DRK PHOTO. 33.27b: © John Bracegirdle/ Masterfile. 33.2S,,: © Art Wolfe. 33.28b: © Anup Shah/Dembinsky Photo Assoc. 33.28c: © Anup Shah/Dembinsky Photo Assoc.

>Bd; © Stan Osolinsky/Dembinsky Photo Assoc. 33.31a: € Dembinsky Photo Assoc. 33.31/' : £9 Tim Davis/Photo Researchers, Inc. J3 Jlc © John Downer/Planet Earth Pictures.

Chapter 34 Opener: © D. Cavagnaro/Visuals Unlimited. 34.3a: © Jan Tove Johansson/Planet Earth Pictures. 34.3b: © R. Calentine/Visuals Unlimited. 34.4a: © Joyce Photographies/Photo Researchers, Inc. 34.4b: © Renee Lynn /Photo Researchers, Inc. 34.4c: © C. K. Lorenz/The National Audubon Society Collection/Photo Researchers, Inc. 34.7: © Biophoto Associates/Photo Researchers, Inc. 34.9a,b: © Phil Gates, U. Durham/BPS. 34.9c: © Biophoto Associates/Photo Researchers, Inc. 34.9d: © Jack M. Bostrack/Visuals Unlimited. 34.9e: ©John D. Cunningham/Visuals Unlimited. 34.9/' : © J. Robert Waaland/BPS. 34.11b, 34.14: © J. Robert Waaland/BPS. 34.16a: © Jim Solliday/BPS. 34.16b: © Microfield Scientific LTD/Photo Researchers, Inc. 34.16c: © Ray F. Evert, U. Wisconsin, Madison. 34.16d: © John D. Cunningham/Visuals Unlimited. 34.18a left: © Cabisco/Visuals Unlimited. 34.18a right: © J. Robert Waaland/ BPS. 34.18b left: © Cabisco/Visuals Unlimited. 34.18b right: © J. Robert Waaland/BPS. 34.20: ©J. N. A. Lott/BPS. 34.21: ©Jim Solliday/BPS. 34.22: © Phil Gates, U. Durham/ BPS. 34.23b: © Thomas Eisner, Cornell U. 34.23c: © C. G. Van Dyke/Visuals Unlimited.

Chapter 35 Opener: © Patti Murray/Animals Animals. 35.5: Brentwood, B., and J. Crenshaw, 1978. Planta 140:111-120. 35.6: © Ed Reschke/Peter Arnold, Inc. 35.9a: © David M. Phillips/Visuals Unlimited. 35.13: © M. H. Zimmermann.

Chapter 36 Opener: © J. H. Robinson/The National Audubon Society Collection/ Photo Researchers, Inc. 36.1: © Inga Spence/ Tom Stack & Assoc. 36.4: © Kathleen Blan-chard/Visuals Unlimited. 36.6: © Hugh Spencer/Photo Researchers, Inc. 36.8: © E. H. Newcomb and S. R. Tandon/BPS. 36.10: © Gilbert S. Grant/Photo Researchers, Inc. 36.11: © Milton Rand/Tom Stack & Assoc.

Chapter 37 Opener: © Jeremy Wood-house/DRK PHOTO. 37.4: © Tom J. Ul-rich/Visuals Unlimited. 37.5: ©John East-cott, Yva Momatiuk/DRK PHOTO. 37.6:

© J. N. A. Lott/BPS. 37.8: © J. A. D. Zee-vaart, Michigan State U. 37.13: © Ed Reschke/Peter Arnold, Inc. 37.16a: © Biophoto Associates/Photo Researchers, Inc. 37.19: © T. A. Wiewandt/DRK PHOTO. 37.22: Dr. Eva Huala, Carnegie Institution of Washington.

Chapter 38 Opener: © C. C. Lockwood/Animals Animals. 38.1 lower: © J. R. Waaland/BPS. 38.1 upper: © Jim Solliday/BPS. 38.2: © Oliver Meckes/Science Source/Photo Researchers, Inc. 38.3: © Stephen Dalton/The National Audubon Society Collection/Photo Researchers, Inc. 38.5: © Bowman, J. (ed.), 1994. Arabiopsis: An Atlas of Morphology and Development. Springer-Verlag, New York. Photo by S. Craig & A. Chaudhury. 38.9a: © C. P. George/ Visuals Unlimited. 38.9b: © Tess & David Young/Tom Stack & Assoc. 38.17a: © Nigel Cattlin, Holt Studios International/Photo Researchers, Inc. 38.17b: © Jerome Wexler/ The National Audubon Society Collection/ Photo Researchers, Inc.

Chapter 39 Opener: Agricultural Research Service, USDA. 39.2: © D. Cavagnaro/Visu-als Unlimited. 39.4: © Stan Osolinski/Dembinsky Photo Assoc. 39.7: © Thomas Eisner, Cornell U. 39.8: © Adam Jones/Dembinsky Photo Assoc. 39.9: ©J. N. A. Lott/BPS. 39.10, 39.11: © Richard Shiell. 39.12: © Ja-nine Pestel/Visuals Unlimited. 39.13: © Chip Isenhart/Tom Stack & Assoc. 39.14: © J. N. A. Lott/BPS. 39.15: © Robert & Linda Mitchell. 39.16: © Budd Titlow/Visu-als Unlimited.

Chapter 40 Opener: © S. Asad/Peter Arnold, Inc. 40.3a,b: © Biophoto Associates/Science Source/Photo Researchers, Inc. 40.3c: © G. W. Willis/BPS. 40.4a: © Cabisco/Visuals Unlimited. 40.4b: © Biophoto Associates/Science Source/Photo Researchers, Inc. 40.4c: © Cabisco/Visuals Unlimited. 40.4a": © David M. Phillips/Visuals Unlimited. 40.10a: © B. & C. Alexander/Photo Researchers, Inc. 40.10b: © Timothy Ransom/ BPS. 40.12: © Auscape (Parer-Cook)/Peter Arnold, Inc. 40.16: © G. W. Willis/BPS. 40.17a: © Stephen J. Kraseman/DRK PHOTO. 40.17b: © Jim Roetzel/ Dembinsky Photo Assoc.

Chapter 41 Opener: © R. D. Fernald, Stanford U. 41.6a: © Associated Press Photo. 41.6b: © Bettman/CORBIS. 41.14a: Courtesy of Gerhard Heldmaier, Philipps University.

Chapter 42 Opener: © Nik Wheeler. 42.1a: © Biophoto Associates/Photo Researchers, Inc. 42.1b: © Brian Parker/Tom Stack & Assoc. 42.1c: © Thomas Eisner, Cornell U. 42.2: © Patricia J. Wynne. 42.3: © David M. Phillips/Science Source/Photo Researchers, Inc. 42.5: © Fred Bavendam/Minden Pictures. 42.6: © David T. Roberts, Nature's Images/The National Audubon Society Collection/Photo Researchers, Inc. 42.7a: © Mitsukaj Iwago/Minden Pictures. 42.7b: ©

Johnny Johnson/DRK PHOTO. 42.12 inset: © P. Bagavandoss/Photo Researchers, Inc. 42.16: © CC Studio/Photo Researchers, Inc.

Chapter 43 Opener: © Dave B. Fleetham/ Tom Stack & Assoc. 43.5 inset: Courtesy of Richard Elinson, U. Toronto. 43.24a: © C. El-deman/Photo Researchers, Inc. 43.24b: © Nestle/Photo Researchers, Inc. 43.26: © S. I. U. School of Med./Photo Researchers, Inc.

Chapter 44 Opener: © Associated Press Photo. 44.4: © C. Raines /Visuals Unlimited.

Chapter 45 Opener: Courtesy of Grace Sours, ATE 45.4 left: © R. A. Steinbrecht. 45.4 right: © G. I. Bernard/Animals Animals. 45.6, 45.12: © P. Motta/Photo Researchers, Inc. 45.15b: © S. Fisher, U. California, Santa Barbara. 45.19a: © Dennis Kunkel, U. Hawaii. 45.22: © Omikron/Science Source/Photo Researchers, Inc. 45.26: © Joe McDonald/ Tom Stack & Assoc.

Chapter 46 Opener: From Harlow, J. M.,

1869. Recovery from the passage of an iron bar through the head. Boston: David Clapp & Son. 46.14: David Joel, courtesy of Bio-logic Systems Corp. 46.16: © Wellcome Dept. of Cognitive Neurology/Science Photo Library/Photo Researchers, Inc.

Chapter 47 Opener: © AFP/CORBIS. 47.2: © P. Motta/Photo Researchers, Inc. 47.5 upper: © CNRI/Photo Researchers, Inc. 47.5 center: © G. W. Willis/BPS. 47.5 lower: © Michael Abbey/Photo Researchers, Inc. 47.7: © Frank A. Pepe/BPS. 47.12: Courtesy of Jesper L. Andersen. 47.14: © Skip Moody/ Dembinsky Photo Assoc. 47.18a: © G. Mili. 47.18b: © Robert Brons/BPS. 47.22a: © Ken Lucas/Visuals Unlimited. 47.22b: © Fred McConnaughey/The National Audubon Society Collection/Photo Researchers, Inc.

Chapter 48 Opener: © Darrell Gulin/Tony Stone Images. 48.1a: © Ed Robinson/Tom Stack & Assoc. 48.1b: © Robert Brons/BPS. 48.1c: © Tom McHugh/Photo Researchers, Inc. 48.3: © Eric Reynolds/Adventure Photo. 48.5b: © Skip Moody/Dembinsky Photo Assoc. 48.5c: © Thomas Eisner, Cornell U. 48.9: © Walt Tyler, U. California, Davis. 48.12 left inset: © Science Photo Library/Photo Researchers, Inc. 48.12 right inset: © P. Motta/Photo Researchers, Inc. 48.15: © Fred Brummer/DRK PHOTO.

Chapter 49 Opener: © Norbert Wu / DRK PHOTO. 49.9: © Geoff Tompkinson/Photo Researchers, Inc. 49.11: © Dennis Kunkel, U. Hawaii. 49.14a: © Chuck Brown/Science Source/Photo Researchers, Inc. 49.14b: © Biophoto Associates/Science Source/ Photo Researchers, Inc. 49.15: After N. Campbell, 1990. Biology, 2nd Ed., Benjamin Cummings Publishing Co. 49.16a: © NYU Franklin Research Fund/Phototake. 49.17b: © CNRI/Photo Researchers, Inc.

Chapter 50 Opener: © Bettmann/CORBIS. 50.1a: © Mike Barlow/Dembinsky Photo Assoc. 50.1b: © Jim Battles/Dembinsky Photo Assoc. 50.1c: © James Watt/Animals Animals. 50.1d: © Tom Walker/Visuals Unlimited. 50.6: © Ken Greer/Visuals Unlimited. 50.7: © Carl Purcell/Photo Researchers, Inc. 50.8a: © David Roberts/Nature's Images/Photo Researchers, Inc. 50.8b: © Gary Milburn/Tom Stack & Assoc. 50.11: © Dennis Kunkel, U. Hawaii. 50.22: © Jackson/Visuals Unlimited. 50.23 left: © Richard Shiell/ Animals Animals. 50.23 right: © Katsutoshi Ito/Nature Productions. 50.24: © L. Kiff/Visuals Unlimited.

Chapter 51 Opener: © Michael Fogden/ DRK PHOTO. 51.1a: © Brian Kenney/Plan-et Earth Pictures. 51.2b: © Rod Planck/Photo Researchers, Inc. 51.8: From Kessel, R. G., and R. H. Kardon, 1979. Tissues and Organs. W. H. Freeman, San Francisco. 51.9: © John Cancalosi/DRK PHOTO. 51.13: © Lise Bankir, INSERM Unit, Hopital Neck-er, Paris.

Chapter 52 Opener: © Frans de Waal, Emory U. 52.1: © Bill Beatty/Visuals Unlimited. 52.4: © Marc Chappell, U. California, Riverside. 52.6: © Nina Leen/TimePix. 52.13: © Francois Savigny/Animals Animals. 52.19: © Fritz Polking/Dembinsky Photo Assoc. 52.25: © Jonathan Blair/ Woodfin, Camp & Assoc.

Chapter 53 Opener: © Anup and Monoj Shah/A Perfect Exposure. 53.4b: © Dominique Braud/Tom Stack & Assoc. 53.5: © John Alcock, Arizona State U. 53.6: Courtesy of Arild Johnsen, University of Oslo. 53.7: © Anup Shah/Planet Earth Pictures. 53.10: © Art Wolfe. 53.11: © D. Houston/ Bruce Coleman, Inc. 53.12: © John Gerlach/ Dembinsky Photo Assoc. 53.13: © Andrew J. Martinez/The National Audubon Society

Collection/Photo Researchers, Inc. 53.14: © Art Wolfe. 53.16: © Jonathan Scott/Planet Earth Pictures.

Chapter 54 Opener: © Pat Anderson/Visuals Unlimited. 54.1a: © Breck P. Kent/Animals Animals. 54.1b: © John Gerlach/Visu-als Unlimited. 54.2: © Ted Mead/Woodfall Wild Images. 54.9a: © Frans Lanting/Min-den Pictures. 54.9b: © Art Wolfe. 54.10: © Adam Jones/Dembinsky Photo Assoc. 54.11a: © Art Wolfe/The National Audubon Society Collection/Photo Researchers, Inc. 54.11b: © Frans Lanting/Photo Researchers, Inc. 54.15: © John R. Hosking, NSW Agriculture, Australia.

Chapter 55 Opener: © Maximilian Stock Ltd./Science Photo Library/Photo Researchers, Inc. 55.5: © Tom Brakefield/DRK PHOTO. 55.8a: © Thomas Eisner and Daniel Aneshansley, Cornell U. 55.8b: © David J. Wrobel/BPS. 55.9, 55.10: © Lawrence E. Gilbert/BPS. 55.11: © Callanan/Visuals Unlimited. 55.12: © Stephen G. Maka/DRK PHOTO. 55.13: © Patti Murray/Animals Animals. 55.14: © Daniel Janzen, U. Pennsylvania. 55.15a: © Merlin D. Tuttle/Bat Conservation International /Photo Researchers, Inc. 55.15b: © Roger Wilmschurst/Photo Researchers, Inc. 55.16: © Dr. Edward S. Ross, California Academy of Sciences. 55.17a: © Darrell Gulin/Dembinsky Photo Assoc. 55.17b: © Gilbert Grant/Photo Researchers, Inc. 55.18: © Jan Tove Johansson/Planet Earth Pictures. 55.19: © Jeff Mondragon. 55.21: After Begon, M., J. Harper, and C. Townsend, 1986. Ecology, Blackwell Scientific Publications.

Chapter 56 Opener: © Tom Bean/DRK PHOTO. 56.9a: © Joe McDonald/Tom Stack & Assoc. 56.9b: © Inga Spence/Tom

Stack & Assoc.

Chapter 57 Opener: © Dave Watts/Tom Stack & Assoc. 57.3: © Elizabeth N. Orians. 57.7: Courtesy of E. O. Wilson. Tundra, upper: © Tom & Pat Leeson/DRK PHOTO. Tundra, lower: © Elizabeth N. Orians. Boreal forest, upper: © Carr Clifton/Minden Pictures. Boreal forest, lower: © Ted Mead/ Woodfall Wild Images. Temperate deciduous forest: © Paul W. Johnson/BPS. Temperate grasslands, upper: © Robert and Jean Pol-lock/BPS. Temperate grasslands, lower: © Elizabeth N. Orians. Cold desert, upper: © Edward Ely/BPS. Cold desert, lower: © Art Wolfe. Hot desert, left: © Terry Donnelly/ Tom Stack & Assoc. Hot desert, right: © Ted Mead/Woodfall Wild Images. Chaparral: © Elizabeth N. Orians. Thorn forest: © Nick Garbutt/Indri Images. Savanna: © Tim Davis/ The National Audubon Society Collection/Photo Researchers, Inc. Tropical deciduous forest: © Donald L. Stone. Tropical evergreen forest: © Elizabeth N. Orians. 57.14: © Gary Bell/Masterfile.

Chapter 58 Opener: © D. Pratt (Oil Painting), Bishop Museum. 58.4: © Kevin Schafer/Tom Stack & Assoc. 58.5a: © Elizabeth N. Orians. 58.8a: © Peter Bichier/ Smithsonian Migratory Bird Center. 58.8b: © Inga Spence/Visuals Unlimited. 58.10: Richard Bierregaard, Courtesy of the Smithsonian Institution, Office of Environmental Awareness. 58.11a: © Frans Lanting/Min-den Pictures. 58.11b: © John S. Dunning/ The National Audubon Society Collection/Photo Researchers, Inc. 58.12: © Adri-enne Gibson/Animals Animals. 58.13: © Ed Reschke/Peter Arnold, Inc. 58.14: © David Boynton. 58.15a: © John Gerlach/DRK PHOTO. 58.16a: © Richard P. Smith. 58.17: © Frans Lanting/Photo Researchers, Inc. 58.18: © Nick Garbutt/Indri Images.

Numbers in boldface italic refer to information in an illustration, caption, or table.

A band, 836, 837 Abdomen, of crustaceans, 571 Abiotic environment, 947 ABO blood group system, 187 Abomasum, 902, 903 Abortion, 747

Abscisic acid, 289, 627, 647, 660 Abscission, 655, 658 Abscission zone, 655 Absorption spectrum, 140 Absorptive period, 903-904, 905 *Abudeiduf saxatilis*, 956 Abyssal zone, 1026 Acacia, 1023

A. comigera, 984 Accessory fruits, 525 Accessory pigments, 141 Accessory sex organs, 737 Acclimatization, 700, 701 Accommodation, 808 Acellular organisms, 240

slime molds, 496-497 *Acetabularia*, 492 Acetaldehyde, 129, 230 Acetate, 122, 223 Acetic acid, 29 Acetylcholine, 289, 787, 789

acetylcholinesterase and, 785-786

actions of, 790

autonomic nervous system and, 821, 822

heart pacemaker cells and, 873

in neuromuscular junction, 785

in smooth muscle contraction, 834, 835 Acetylcholine receptors, 283, 786,

787, 788 Acetylcholinesterase, 112,

785-786, 787, 791 Acetyl coenzyme A, 122, 323, 124, 133

anabolic interconversions, 131-132 Acetyl group, 889 Acidophiles, 473 Acid precipitation, 1004, 1005 Acids, 28-30 Acoelomates, 544, 545

Acorn worms, 581-582

Acropora, 551, 737

Acrosomal process, 753, 755

Acrosomal reaction, 753, 754

Acrosome, 740, 753

Actin, 72-73

in cytokinesis, 164 in microfilaments, 833 in muscle contraction,

833-834, 836, 837-839 in myofibrils, 836, 837 structure of, 837 See also Microfilaments

Actinomyces israelii, 472

Actinomycetes, 472, 640

Actinopods, 496

Action potentials, 777

in cardiac muscle, 873-874 conduction of, 781-783 generation of, 780-781 neurotransmitter release and, 786, 787 refractory period, 780 rising phase, 780 saltatory conduction, 784-785 self-regenerating, 783 in sensory transduction, 796 skeletal muscle contraction

and, 838, 839 smooth muscle contraction and, 834, 835

Action spectrum, 140-141

Actionsphaerium eichorni, 496

Activating enzymes, 226, 227

Activation, G protein-mediated, 284

Activation energy, 102-103, 104-105

Active sites, 104,105,106

Active transport described, 88-89 primary and secondary, 89-90 proteins involved in, 89 in uptake by plants, 621-622, 624

Acute toxins, 982

Adam's apple, 857

Adaptation (evolutionary), 4, 7, 395, 396

Adaptation (sensory), 797

Adenine, 47, 48

cytokinins and, 658 in DNA, 203, 204, 205

Adenosine, 279, 790, 826

Adenosine deaminase deficiency,

347 Adenosine diphosphate (ADP),

100-101, 128 Adenosine monophosphate

(AMP), 101, 201 Adenosine triphosphate (ATP) allosteric regulation of metabolism, 133 coupling of exergonic and endergonic reactions, 101-102 creation in mitochondria, 68 food energy and, 887 functions of, 49,101 invested in glycolysis, 118-119 in primary active transport,

89,90 protein kinases receptors and,

283 structure of, 102 used in photosynthesis, 147,

148 See also ATP synthesis Adenovirus, 240 Adenylyl cyclase, 287 Adipose tissue, 696, 697, 706 ADP. See Adenosine diphosphate Adrenal cortex, 719, 725-726 Adrenal gland, 714, 719, 724-726,

882, 883 Adrenaline. See Epinephrine Adrenal medulla, 719, 725 Adrenergic receptors, 725 Adrenocorticotropin (ACTH),

718, 719, 720, 725, 726 Adrenocorticotropin-releasing

hormone, 721, 726 Adriamycin, 347 Adult-onset diabetes, 729 Adult stage, in insects, 717 Adventitious roots, 605 Aequopora victoriana, 317 Aequorea victoria, 218 Aequorin, 218 Aerenchyma, 687, 688 Aerobic respiration, 115

emergence of, 4 energy yields, 130 evolution of, 454-455 in prokaryotes, 464 Afferent blood vessels, 854

arterioles, 916, 919-920, 922 Afferent nerves, 815 Aflatoxins, 536

African Americans, sickle-cell

anemia and, 333 African ass, 1009 African chameleon, 590 Agalynchnis calcarifer, 738 Agar, 491

Agaricus bisporus, 538 Agassiz, Louis, 569 Agaves, 968, 969 Age distribution, 961-962 Agelaius, 414 Aggregate fruits, 525

Aggressive behavior, 927 Aging, telomerase and, 264 Agnathans, 578, 585 Agnosias, 819 Agriculture

biotechnology and, 325-327

ecological components, 998

fertilizers, 639

habitat destruction, 1034

integrated pest management, 998-999

liming, 639

methods of vegetative reproduction, 677

origins of, 599-600

photosynthetic efficiency and, 136

salinization of land, 688

soil and, 638, 639 *Agrobacterium tumefaciens*, 316, 327, 469 *Agrostis*, 689

AIDS, 372-375, 533, 748-749, 750 Air breathing

evolutionary transitions to, 869

See also Breathing Air capillaries

in birds, 855

in insects, 853 Air pollution, lichens and, 540 Air sacs, 854, 855, 856 Alanine, 37, 224 Albatrosses, 940-941 Albinism, 185

Albizia, 674 Albumin, 881 Alcoholic fermentation

overview of, 129, 130

Pasteur's study of, 114 Alcoholism, edema and, 877 Alcohols, 32 Aldehydes, 32

Aid.

Aldolase 120

Aldosterone 725 922

Aleurone layer 6 ; ; 651

Algae

blown 4— 188 489

rod. 477 491,495 496 ibo Green algae Alginate acid, 489 *Alisteria scapularis*, 592 Alkaloids 683 Alkaptonuria, 220, 331

Allantoic 767, 768 Allard, H. A., 671 Allele frequencies

calculating, 399-400

Hardy-Weinberg equilibrium, 400-402

natural selection and, 405 Alleles, 180

codominance in, 187

defined, 398

incomplete dominance in, 186-187

Mendel's law of independent assortment, 182-183

Mendel's law of segregation, 180-182

multiple, 186

mutant, 186

neutral, 408

pleiotropic, 187-188

polymorphic, 186

sexual reproduction and, 408

wild type, 186 Allele-specific cleavage testing, 341, 342 Allele-specific oligonucleotide hybridization, 341, 342 Allergic reactions, dynamics of inflammation, 876-877 Alligator mississippiensis, 167, 590 Alligators, 167, 590 Allomyces, 534 Allopatric speciation, 414-417 Allopolyploidy, 417 Allosteric enzymes, 109-110, 111, 132-134 Allosteric regulation evolution and, 133-134 of metabolic pathways, 110, 111, 132-134 Allosteric site, 110 Alpacas, 600 Alper, Tikva, 334 Alpex lagopus, 707 Alpha (a) helix, 38-39 of DNA, 48, 203, 204, 205 Alpine tundra, 1016 Alternate splicing, 276 Alternation of generations, 489-190, 501 Altruism, 953, 954-956 Aluminum, plant tolerance to, 689 Alu sequence family 264, 448 Alvarez, Luis, 384 Alveolata, 477, 484-487 Alveoli in Alveolata, 484 of lungs, 857, 859 in paramecia, 486 Alzheimer's disease, 826 Amacrine cells, 810 Amanita, 538 AmboreUa, 525, 526 Amblyrhynchus cristatus, 704 Ambulocetus, 385 Amensalism, 975, 982-983 American chestnuts, 1037 American elms, 1037 Amines, 31 Amino acid residues, 36 Amino acids anabolic interconversion, 131 in animal nutrition, 889-891 assembly into polypeptides, 228-230 essential, 889, 890 functional groups, 31 genetic code, 223-225 isomeric forms, 32 as neurotransmitters, 790 peptide linkages, 37-38 side chains, 36-37 tRNA specificity, 225-226, 227 Amino acid sequences analyzing change in, 440-441 evolutionary change in cytochrome c, 441, 442-443 Aminoacyl-tRNA-synthetases, 226, 227 Amino groups, 31, 37-38 Aminopeptidase, 901 Amino sugars, 46 Ammonia excretion of, 912 nitrogen fixers and, 640, 641 Ammonotelic animals, 912

Amniocentesis, 768

Amnion, 765, 767, 768

Amnionic cavity, 767, 768

Amniotes, 588-589

Amoebas, 476, 495[^]96 movement in, 833 pseudopods, 480 shells of, 483

AMPA receptor, 789, 790, 791

Amphibians (Amphibia), 578 adaptations for water conservation and salt excretion, 917-918 evolution of, 587 gastrulation in, 761-762 general characteristics, 587 mating behavior, 737 recent declines in, 587-588 three-chambered heart, 869

Amphignathodon, 427

Amphipods, 11-12, 13, 569

Amphisbaenians, 589

Ampicomplexans, 484-485

Amplexus, 737, 738

Amplitude, of cycles, 673

Ampullae, 802

Amylase, 898, 901

Amylose, 46

Anabena, 470

Anabolic pathways

integration with catabolic

paths, 132 interconversions, 131-132

Anabolic reactions, 96

Anabolic steroids, 727 Anacharis, 141

Anaerobic bacteria, 146 Anaerobic metabolism

in diving mammals, 884

in prokaryotes, 464 Anaerobic respiration, 115. See

also Fermentation Anaphase

meiosis, 168, 169, 171

mitosis, 163, 164 Ancestral traits, 427, 428 Anchor cells, 302, 303 Andansonina grandieri, 1041 Androgens, 719, 726, 727

Androstenedione, 725 Anemia, 892 Anencephaly, 766 Aneuploidy, 172-173, 335 Angelman syndrome, 339 Angina, 289

Angiogenesis, in cancer, 343 Angiosperms (Angiospermae), 434, 501, 517. See also Flowering plants Angiotensin, 882, 922

Angular gyrus, 828, 829 Animal-animal mutualisms, 984 Animal cells

cytokinesis, 164, 265

extracellular matrix, 76-77

gap junctions, 292

structure and organization, 60

See also Cells Animal classification, 434, 435 Animal cloning, 297-299 Animal communication, 935-937 Animal defense systems

cells and proteins in, 354-355

distinguishing self and non-self, 353

evolution of, 372

innate, 353-354, 355-358

invertebrate, 372

See also Immune system Animal development

cleavage, 756-759

extraembryonic membranes, 767-768

gastrulation, 759-765

human pregnancy and birth, 769-771

interactions at fertilization, 753-756

neurulation, 765-767

overview of, 752 Animal hemisphere, 301, 755,

756, 757 Animalia, 10, 435, 543-544, 578 Animal nutrition, 886

carbon skeletons and essential amino acids, 889-891

control and regulation of fuel metabolism, 903-905

energy measurements, 886-887

energy storage in the body, 888

feeding adaptations, 893-894, 895

food intake regulation, 906

mineral elements in, 890, 891

nutrient deficiency diseases,

892-893 undernourishment and

overnourishment, 888-889 vitamins, 891-892 See also Digestion Animal physiology defined, 693 homeostasis, 694-695 properties of regulation,

698-699 thermoregulation, 700-710 Animals

ancestral lineage, 543-544 body plans, 545-546 body temperature and thermoregulation, 700-710 characteristic traits, 544 determining evolutionary relationships in, 544-545 evolution of bilateral symmetry, 552 evolution of segmented bodies, 558 phylogeny, 545 protostome-deuterostome lineage split, 552-553 shared derived traits, 544 temperature sensitivity, 700 Animal societies, evolution of,

956-958 Animal viruses, reproductive

cycle, 242-243 Anions, 24

soil pH and, 640 Anisogamous life cycle, 493 Annelids (Annelida), 558-559 circulatory system, 868 general characteristics, 574 metanephridia, 914, 915 nervous system, 774 subgroups and number of living species, 578 Annotation, 262 Annual plants, 671

in chaparral biomes, 1022 in deserts, 685 survivorship curves, 963 Annual rings, 616, 627 Anogential cancers, 343 Anopheles, 413, 485 Anorexia nervosa, 889 Antarctica, lichens in, 540 Antelopes, 421 Antennapedia mutation, 307 Antennarius commersonii, 586 Antenna systems, 142 Anterior pituitary development of, 717 hormones and targets of, 728, 719-720 hypothalamus and, 720-721 negative feedback regulation

of, 721-722 ovarian and uterine cycles and, 743-745 Anterior-posterior axis, determination of, 756 Anterior-posterior patterning,

766 Anther, 666 Antheridia, 502

ferns, 514

mosses, 506

nontracheophytes, 504, 505 Anthers, 521, 522 Anthocerophyta, 501, 505-506 Antkoceros, 506 Anthoxanthm odoratum,

418 Anthozoans (Anthozoa),

550-551, 578 Anthrax, 467 Anthropoids, 595-597 Antibiotic resistance, 239

as a genetic marker, 315, 317, 318

R factors, 248-249 Antibiotics

from actinomycetes, 472

inhibition of translation, 230

intestinal bacteria and, 902

prokaryote cell walls and, 464 Antibodies, 187, 354

classes of, 363

class switching, 371

defined, 355

evolution of, 372

functional structure, 362, 363

genetic basis of diversity in, 368-371

IgG class, 363-364

overview of, 359

polyclonal, 364

specificity of, 358

See also Immunoglobulins Anticodons, 225, 228 Antidiuretic hormone (ADH), 717-719, 923. See also Vasopressin Antifreeze proteins, 690 Antigen-binding site, of

immunoglobulins, 362, 363 Antigenic determinants, 358, 359 Antigen-presenting cells, 361,

366, 367, 367 Antigens, 187, 358 Antihistamines, 372, 877 Antiparallel strands, DNA, 203 Antipodal cells, 666, 667 Antiport transporters, 89 Antisense RNA, 322 Antitranspirants, 628 a-1-Antitrypsin, 326 Ants, 574, 984. See also Hymenoptera Anurans, 587 Anvil, 802, 803 Aorta, 868

in reptiles, 869

stretch receptors in, 883 Aortic bodies, 864, 884 Aortic valve, 871 Apes, 595

phylogeny, 431 Aphasia, 828 Aphids, 572, 984

parthenogenesis in, 733

in plant phloem experiments, 629 Aphytis, 976, 977 Apical buds, 605 Apical cell division, 506 Apical complex, 484

Apical dominance, 655, 656 Apical hook, 659 Apical meristems

primary meristems formed by, 612-613

root, 612, 613

shoot, 612, 614

tissue culturing, 677

transition to flowering, 670-671 Apicomplexa, 477 Apis mellifera, 573 Aplysina fistularia, 547 Apomixis, 676 Apoplast, 623, 624, 631, 632 Apoptosis, 173-174, 302-304

in lymphocytes, 361

oncogenes and, 344 Appendages, in arthropods, 567 Appendicular skeleton, 843 Appendix, 897, 902 Apples, 525

Aptenodytes patagonicus, 738 Apterygota, 572 Aquaporins, 79, 88, 923 Aquaspmillum sinosum, 461 Aquatic animals

effects of temperature on gas exchange in, 850-851

excretion of ammonia, 912
 gastrovascular cavity in, 867
 partial pressures of carbon dioxide and, 852 Aquatic biogeography,
 1026-1027 Aquatic communities, pyramids of energy and biomass, 997 Arabidopsis, 646
 brassinosteroid-deficient mutants, 660-661
 costs of herbicide resistance, 407
 organ identity genes, 304, 305
 phototropic mutants, 662, 663
 phytochrome genes, 662
 protein kinase cascades and, 287
 sodium transport mutant, 688 Arabinocytosine, 347 Arachidonic acid, 891 Arachnids (Arachnida), 569, 570,
 578 Arboviruses, 242 Arcelin, 684 Archaea, 9, 10
 cell wall characteristics, 463
 chemoautotrophs, 464
 domain, 459, 460, 461
 evolutionary relationships, 467, 468
 lateral gene transfer and, 468
 major groups, 472, 473-474
 membrane lipids, 473
 shared characteristics, 472-473 Archaeobacteria, 459 Archaeopteryx, 591 Archaezoa," 479-480 Archegonia, 502
 ferns, 514
 nontracheophytes, 504, 505 Archenteron, 760, 761
 Arctic animals, strategies for decreasing heat loss, 706-707 Arctic fox, 707 Arctic ground squirrel, 594 Arctic tern, 7
 Ardipithecines, 597 Area phylogenies, 1008, 1009 Arginine, 37, 224, 683 Argyroxiphium sandwichense, 422 Armadillidium
 vulgare, 570 Armillariella mellea, 529 Army ants, 955 Arothron
 A. meleagris, 577
 A. parda, 907 Arrhythmias (cardiac), 874 Arrow worms, 565-566, 578 Artemia, 911, 912 Arteries
 atherosclerosis, 878-879
 defined, 868
 elastic and muscle fibers in, 875-876
 See also Vascular system Arterioles
 afferent, 916, 919-920, 922
 autoregulatory mechanisms and, 882
 defined, 868
 elastic and muscle fibers in, 875-876
 hormonal and neural control of, 883
 See also Vascular system Arthritis, rheumatoid, 372 Arthrotrichs, 532 Arthropods (Arthropoda), 567
 body plan, 564, 568
 chelicerates, 569

chemosensation in, 797

crustaceans, 569-571

dorsal-ventral axis determination, 309

dorsal-ventral development, 543

exoskeleton, 567, 568, 842-843

open circulatory system, 867

trilobites, 568

uniramians, 571-574

visual system, 807 Artificial chromosomes, 316, 349 Artificial immunity, 360 Artificial insemination, 748 Artificial selection, 397, 933 Asci, 535 Ascidiacea, 582 Asclepias syriaca, 685 Ascocarp, 535 Ascomycetes (Ascomycota), 530, 533 Ascorbic acid, 891, 892 Ascospores, 535-536 Asexual reproduction

in animals, 732, 733-734

in diatoms, 488

in fungi, 532-533

mitosis and, 165

in paramecia, 486-487

in plants, 665-666, 676-677

in protists, 482 Ashkenazic Jews, 341

Asparagine, 37, 224 Aspartic acid, 37, 224 Aspens, 676 Aspergillus, 536 Aspirin, 680

effect on fevers, 709 Assisted reproductive technologies (ARTs), 748-750 Association cortex, 819 Associative learning, 827 Associative mating, 404 Asteroidea, 578, 581 Astrocytes, 776 Asymmetric carbon, 32 Asymmetry, 546 Atacama Desert, 686 Ateles, 431, 1036 Atherosclerosis, 878-879 Athletes

anabolic steroids and, 727

muscle fiber types and, 840-841 Atmosphere

circulation patterns, 992, 993

conditions at the origin of life, 451-452

description of, 1000

global warming, 1003

oxygen and evolution, 380, 382, 451-452, 466

temperature regulation, 1000-1001 Atmospheric circulations, 992, 993 Atomic mass, 17-18, 19 Atomic mass number, 18 Atomic mass unit, 17 Atomic number, 19 Atomic weight, 19 Atoms, 8

atomic number, 18

charge, 18

chemical reactions, 25-26

component particles, 17-18

electron behavior in bonding, 19-20

elements, 18

isotopes, 18

mass, 17-18, 19

mass number, 18

weights and sizes, 29 ATR See Adenosine triphosphate ATP synthase, 127, 129, 345, 146 ATP synthesis
 aerobic respiration, 115, 130
 anaerobic respiration, 115
 chemiosmosis, 127-129, 145-146
 citric acid cycle, 122, 123, 124, 130
 fermentation, 129, 130
 glucose metabolism, 115, 130
 glycolysis, 117, 118-119, 120, 223, 230
 oxidative phosphorylation, 125-129
 photophosphorylation, 138, 142, 145-146
 respiratory chain, 118, 125, 126-129, 230
 substrate-level phosphorylation, 120
 Atrial natriuretic hormone, 719
 -r Atrioventricular \al\os s~i. S72 Atripkx hili»: s " ss Atrium
 human heart and cardiac cy « 3 J72, 873-874
 in lungsh, B
 in three-chambered hearts, 869
 in two-chambered hearts, ShS Auditor] communication, 936 Auditor) systems, 802-803,
 Australia
 biota of, 1010
 human-caused extinctions in, 1030, 1037
 Australian tiger leech, 559
 Australopithecines, 597, 598
 Austrobaileva, 523
 Autocatalysis, 898, 899, 900
 Autocrine hormones, 723, 714
 Autocrine signals, 280
 Autoimmune diseases, 359, 362
 Autoimmunity, 372
 Autonomic nervous system, 815 control of breathing, 863-864 control of heartbeat, 873 parasympathetic division,
 821-823, 873, 883 regulation of blood pressure,
 882, 883-^884 svmpathetic division, 821-823, 873, 882, 883
 Autonomic reflexes, 903
 Autophagy, 67
 Autopolyploidy, 417
 Autoregulation
 circulatory system, 882 kidney function, 922
 Autosomal dominant diseases, 335

Autosomal recessive diseases, 335

Autosomes, 194

Autotrophs, 635, 886

Auxin

activities of, 647 carrier proteins, 654 in cell differentiation and organ formation, 658 cell walls and, 657 chemical structure, 652 effects on vegetative growth,

655-656 fruit development and, 656 in gravitropism, 654 oligosaccharins and, 660 in phototropism, 653, 654 polar transport, 653-654 receptor proteins, 657-658 synthetic, 656

Auxotrophs, 220

Avery, Oswald, 201

Aves, 435, 578, 591

Avogadro's number, 28

Avr genes, 681

Axes determination

in animal development, 756 in arthropods and vertebrates, 309

Bicoid-Nanos gradients, 306, 307

maternal effect genes and, 305-306

segmentation genes and, 306 Axial filaments, 462, 470 Axial skeleton, 843 Axonemes, 832, 833 Axon hillock, 787-788 Axons, 718, 775 Axon terminal, 775 Azolla, 640

Baboons, 596, 957 "Baby boomers," 961-962 Bacillariophyta, 477, 487-488 Bacillus, 471

B. anthracis, 467

B. subtilis, 156, 463

B. thuringiensis, 326

cell division in, 156

gram staining, 463

nitrogen metabolism and, 455 Bacillus/Bacilli (prokaryote

form), 461 Bacillus toxins, 326 Bacteria

actions of antibiotics, 230

antibiotic resistance, 239, 248-249

capsule, 59

cell wall, 59

chemosynthetic, 635, 998

colonies, 245

conjugation in, 245-246, 247

expression vectors for, 323

genetic transformation, 200-201

genome, 255-256

Gram staining, 463

growing in the laboratory, 245

in guts, 895-896, 902, 903

largest known, 459
 lateral gene transfer and, 468
 major groups, 468¹⁷²
 nitrogen cycle and, 465-466, 642-643
 nitrogen fixing, 640, 641, 642, 983
 as normal flora, 356
 pathogenic, 466-467, 471-472
 photosynthetic, 59
 plasmids in, 248-249
 regulation of gene expression in, 249-254
 restriction endonucleases, 312
 "rock-eating," 3
 strains, 200
 thermophiles, 469
 transduction in, 247
 transformation in, 247
 as transgene hosts, 315
 transposable elements in, 249
 See also Prokaryotes Bacteria (domain), 9, 10, 459, 460, 461
 evolutionary relationships, 467-468 Bacterial artificial chromosomes,
 349 Bacterial conjugation, 245-246, 247
 Bacterial pathogens, 466-467
 chlamydias, 471
 firmicutes, 471⁷² Bacterial toxins, 326, 467 Bacteriochlorophyll, 464 Bacteriophage
 Hershey-Chase experiment, 201-202
 regulation of lytic and lyso-genic cycles in, 254, 255
 structure and life cycle, 201, 241-242
 transduction and, 247 Bacteriorhodopsin, 474 Bacteroids, 641 Bakanae, 651 Baker's yeast, 535 Balanus balanoides, 975, 976
 Baleen whales, 577 Ball-and-socket joint, 845 Bamboo, 676 BamHI, 317
 Banksia integrifolia, 1010 Banting, Frederick, 724 Bark, 613, 615, 617 Barley seeds, 650 Barnacles, 569, 570
 ecological niches, 975, 976 Barometric pressure, 851 Barr, Murray, 274 Barr bodies, 274-275 Barrel cactus, 428, 606 Basal
 body, 75 Basal lamina, 77, 695 Basal metabolic rate
 of endotherms, 705, 706
 energy yields from food and, 887-888 Basement membrane, 77 Base pairing, complementary, 48,
 204, 205, 206, 209 Bases (chemical), 29-30 bas-1 gene, 661 Basidiocarp, 539 Basidiomycetes (Basidiomycota),
 530, 533, 538, 539, 988 Basidiospores, 538, 539 Basidium, 538, 539 Basilar membrane, 803, 804 Basihsaurus, 385 Basophils,
 355, 357 Batesian mimicry, 980, 982 Bats, 594, 595
 echolocation in, 812
 as pollinators, 985 Bay checkerspot butterfly, 965 B cells, 355, 368
 activation, 359, 362-363

antibody diversity and, 369, 370-371

as antigen-presenting cells, 367

in autoimmunity, 372

class switching, 371

clonal deletion, 360-361

clonal selection, 359-360

daughter cells, 360

in lupus, 372

overview of, 354 bcl-2 gene, 304 Beadle, George W., 218, 220 Beagle (ship), 396

Bean weevils, 684

Beavers, 887, 986

Beech trees, 968-969, 2038

Bee-pollinated flowers, 524

Bees, 573, 574

heat production in, 704 sex determination, 192 See also Honeybees

Beeswax, 53

Beetles, 574

Behavior

approaches to studying, 926 circadian rhythms, 937-939 communication, 935-937 genetics of, 932-934 hormones and, 931-932 influence on speciation, 421 inherited, 926-930 orientation and navigation, 940-944 parenting, 956, 968 in thermoregulation, 702 See also Mating behavior; Sexual behavior; Social behavior

Behavioral ecology

costs and benefits of social

behavior, 952-953 defined, 947 evolution of animal societies, 956-958 foraging theory, 949-950 habitat selection, 948-949 mating behavior, 950-952

Behavioral thermoregulation, 702

Beijerinck, Martinus, 240

Belly button, 771

Benign prostate hypertrophy (BPH), 741

Benign tumors, 343

Benson, Andrew, 147

Bent grass, 689

Benthic zone, 1026

Benzer, Seymour, 226

Benzoapyrene, 236

Benzpyrene, 199

Beriberi, 892, 892

Bermuda grass, 605

Best, Charles, 724

Beta blockers, 725
 Beta (B) pleated sheets, 39
 Beta thalassemia, 269-270
 Bicarbonate buffering system, 924
 Bicarbonate ions, 862, 863, 876, 900
 bicoid gene, 305
 Bicoid protein, 306, 307, 308
 Bicoordinate navigation, 941, 943-944
 Bicyclus anynana, 410
 Biennials, 652, 671
 Bilateral symmetry, 546 evolution in animals, 552
 Bilayers, phospholipid, 51, 79-81
 Bile, 899-900, 901
 Bimodal distributions, 406
 Bind in, 753
 Binocular cells, 824
 Binocular vision, 824
 Binomial nomenclature, 433-434
 Bioaccumulation, of toxins, 907 Biodiversity, importance of preserving, 1043 Biogeochemical cycles,
 1001-1005 Biogeographic regions, 1011 Biogeography, 1007
 approaches to understanding species' distribution, 1008-1011 aquatic systems, 1026-1027 area phylogenies, 1008,2009
 biogeographic regions, 1011 continental drift, 1008 ecology and, 1011-1014 human history and, 1028 island model of species
 richness, 1012-1014 terrestrial biomes, 1014-1025 using parsimony in, 1010 vicariant and dispersal distributions, 1009-
 1010 Biological clocks
 circadian rhythms, 673-674,
 937-939 circannual rhythms, 940 molecular, 439, 441 ^42, 940 photoperiodism and, 674, 940 Biological control, life histories
 and, 970-972 Biological detoxification, 907 Biological evolution, 379-380. See
 also Evolution Biological names, binomial
 nomenclature, 433 ^134 Biology
 approaches to the study of
 life, 7 emergent properties, 9 hierarchical classification of
 life, 9-10 public policy and, 14 scientific inquiry, 10-14 Bioluminescence, 218, 484 Biomass, 960, 997 Biomes
 boreal forest, 1017
 chaparral, 1022
 cold deserts, 1020
 geographic distribution, 1025
 hot deserts, 1021
 means of identifying, 1014
 savannas, 1023
 temperate deciduous forests,

1018 temperate grasslands, 1019 thorn forests, 1023 tropical deciduous forests,
1024 tropical evergreen forest, 1025 tundra, 1016 Bioremediation, 689 Biosphere, 8 Biotechnology
agricultural applications,
325-327 medically useful products
from, 323-325 nitrogen fixation and, 641-642 overview of, 322
public concerns with, 327-328
silk protein and, 311
using expression vectors in, 322-323
See also DNA technology Biotic environment, 947 Biotin, 892, 902 Bipedalism, in human evolution,
597 Bipolar cells, 810 Biradial symmetry, 546, 579 Bird-pollinated flowers, 524 Birds
brood parasitism, 395
characteristics of, 592
circadian clock in, 938
circulatory system, 870-871
courtship displays, 926-927, 927
crop milk, 714
DDT and, 907
effects of testosterone on brain function, 932
extraembryonic membranes, 767-767
fertilization in, 738
flightless, 447, 448, 592, 1030
flocking, 953
foregut fermentation in, 446
gaping, 423
gastrulation in, 763-764
homing in, 940-941
imprinting in, 929
lungs and respiratory gas exchange, 849, 854-856
migratory restlessness, 941
nasal salt glands, 912, 913
navigation during migration, 941-943
origin of, 591
parenting behavior, 956
pecking response, 927-928
reproductive behavior, 950-951, 952
reptiles and, 589
sex determination, 194
sexual selection in, 950-951

survivorship curves, 963
 traditional and phylogenetic classification of, 436 Bird songs
 learning and imprinting in, 929-930
 testosterone and, 932 Birth, in humans, 770-771 Birth control pills, 746 Birth defects, neural tube-related, 766 Birth rates
 density-dependent and density-independent, 965-966
 in population dynamics, 962 Bison, 986, 987 Bison bison, 986, 987 1,3-Bisphosphoglycerate (BPG),
 119-120, 121 bithorax gene, 307, 445 Bivalents, 171
 Bivalves (Bivalvia), 560, 562, 578 Black-bellied seedcracker, 406, 407
 Blackbirds, 423 Black bread mold, 534, 535 Black-browed albatrosses, 942 Black cherry trees, 962 Black-tailed rattlesnake,
 822 Black tern, 996 Bladder cancer, 286 Bladders
 of brown algae, 489 urinary, 919 Blades, of leaves, 605 Blastocoel, 756, 758, 763 Blastocysts, 299, 742, 743, 745,
 758, 769 Blastodisc, 757, 763 Blastomeres, 756, 757, 758-759 Blastopore, 764 Blastula, 756 avian, 763 fate maps, 758 in
 gastrulation, 759-764 Bleaching, in photosensitivity,
 805 Blending theory of inheritance,
 177, 180 Blind spot, 809
 Blocks to polyspermy, 754, 755 Blood, 697
 components of, 879, 880 fish countercurrent heat exchanger, 704-705 glomerular filtration, 916 in heat exchange and
 thermoregulation, 702-703, 704, 705, 707 packed-cell volume, 879 plasma, 881-882 platelets, 880-881 red blood cells, 879-
 880 transport of respiratory gases, 860-863 Blood-brain barrier, 776, 877 Blood calcium, hormonal control
 of, 723, 724 Blood cells, 355. See also Red
 blood cells; White blood cells Blood clotting, 95, 880-881 Blood flow
 afferent and efferent, 854 autoregulation of, 882 concurrent, 855 countercurrent flow in fish
 gills, 854, 855 diving reflex and, 884 pulmonary and systemic circuits, 868-871 through the human heart, 871-872 Blood
 glucose
 insulin and, 723-724 regulation of, 904-905 Blood osmolarity, regulating, 923 Blood pH
 oxygen-hemoglobin binding
 properties and, 862 regulation of, 924 Blood plasma, 354, 697
 transport of respiratory gases, 860, 863 Blood pressure, 872, 873
 regulation of, 882-884
 kidney function and, 922-923 Blood sea star, 580 Blood types, 187 Blood vessels
 afferent, 854, 916, 919-920, 922
 anatomy of, 875
 arteries and arterioles, 875-876
 capillaries, 876-877
 efferent, 854
 organization in the kidney, 919-920
 veins, 877-878
 See also Vascular system Bluebells, 433 Bluefin tuna, 704-705 Bluegill sunfish, 949-950 Blue-light receptors, 661, 662-663
 biological clock and, 674
 photoperiodism and, 673 Bluethroats, 952 Blue whales, 970, 972 Blumeria graminis, 532 BMP4, 309 Body burdens, 907 Body
 cavities, 546

evolution of segmentation, 558
 in major protostomate phyla, 574
 types of, 544, 545 Body plans, 545-546
 molluscan, 560
 respiratory gas exchange and, 850
 sponges, 547 Body segmentation, genes involved in, 305-308, 766-767 Body temperature
 acclimatization, 700, 720
 in camels, 693
 classifications of, 700-701
 control and regulation of, 701-710
 environmental temperature and, 700
 metabolic compensation, 700 Bohadschia argus, 580 Bohemian waxwing, 985 Bohr effect, 862 Bolting, 652
 Bombardier beetles, 982 Bombycilla garrulus, 985 Bombykol, 797 Bone, 696, 697
 calcium in, 843
 cells in, 77
 dynamic remodeling, 843-844 Bone marrow
 production of red blood cells, 880, 882
 transplantation, 259 Bonner, James, 672 Bony fishes, 585-586
 adaptations for water conservation and salt excretion, 917
 evolution of jointed fins, 587 Boreal forest, 1017 Boron, 636 Bothus lunatus, 411
 Bottle cells 761 Bottleneck effects, 402 403 Botulism k>7
 Bowman's capsule 916, 917 Brachinus 98 1
 Brachiopods (Brachiopoda), 555, 557 574 578
 Bracket fungi 538 Bracts, 670 671 Brain actions of caffeine in, 279
 blood-brain Kir nor, 776, 877
 v onsdousness ^nA. S29
 development of, 816
 effects ol testosterone and, 932
 functional imaging, 829
 in human evolution, 598
 learning and, 826-827
 memory and, 827-828
 number of neurons in, 814
 sleeping and dreaming, 825-826
 visual information processing, 823-824
 See also Central nervous system; Cerebral cortex; Cerebrum Brain-derived neurotropic factor,
 323 Brain hormone, 716 Brain imaging, 829 Brain stem, 816
 control of breathing, 863-864

reticular system of, 818

in sleep, 826 Branches (plant), 605, 611-612 Branchiostoma californiense, 582 Brassicaceae, 660-661 Brassica oleracea, 399 Brassinolide, 280 Brassinosteroids, 647, 660-661 BRCA1 gene, 345 Bread mold, 218-220 Breast cancer, 345 Breathing

control and regulation of, 863-864

evolutionary transitions to air breathing, 869

return of venous blood and, 879

tidal, 856-857, 858-859

See also Respiratory system Breeding territories, 948, 952 Brenner, Sydney, 263 Brewer's yeast, 535 Brewing industry, use of gib-

berellins in, 650 Briggs, Robert, 297 Bright-field microscopy, 57 Brine shrimp, 911, 972 Bristlecone pines, 603 Brittle star, 580 Broad-winged katydid, 573 Broca's area, 828, 829 Broccoli, 399 Brock, Thomas, 216 Bromeliads, 473 Bronchi, 859

in birds, 854

in mammals, 857, 858 Bronchioles, 857, 858, 859

Brood parasitism, 395

Brown algae, 477, 488-189

Brown fat, 706

Brown lemur, 1041

Brown plant kingdom, 501

Brown \ oleano sponge, 547

Browsers, 595

Brussels sprouts, 399

Bryophyta, 507, 506-507

Bryopsis, 492

Bryozoans (Bryozoa), 556-557,

574, 578, 960 Buccal cavity, 895 Buckwheat, 689 Budding

in animals, 733

in hemiascomycetes, 535 Buds (plant), 605 Buffering, 924 Buffon, George-Louis Leclerc de,

1-2 Bufo americanus, 167 Bulbourethral glands, 739, 740 Bulbs, 676 Bulk flow, 621

mechanisms of, 631

in phloem, 630-632

in roots, 622-624

in xylem, 624-626 Bundle of His, 874 Bundle sheath, 249, 150, 618 Bungarotoxin, 847 Burrowing, 955 Butterflies, 574

metamorphosis, 572, 726, 717

mimicry, 982

parallel evolution in, 427

phenotypic plasticity, 410

population dynamics, 965

sex determination, 194

Cabbage, 399 Cabbage coral, 552 Cacti, 959

biological control of, 971-972

cactus family, 527

root systems, 687

spines of, 686 *Cactoblastis cactorum*, 971-972 Caddisflies, 574 Caecilians, 587 *Caenorhabditis elegans*, 567

apoptosis in, 302-303

genome, 262, 446

induction during vulval development, 302, 303 Caffeine, 279, 290 Caimans, 590 Calcaneus, 843 Calciferol, 891, 892 Calcitonin, 728, 723, 724 Calcium

in animal nutrition, 890, 891

in bone, 843

in long-term potentiation, 827

membrane potential and, 777

NMDA receptors and, 790, 791

in plant nutrition, 636

regulation of, 290

as second messengers, 288-289, 291

in skeletal muscle contraction, 838-839, 840

in smooth muscle contraction, 839

in soils, 638 Calcium-binding protein, 89 Calcium channels, 288-289

in neurotransmitter release, 786, 787

in non-REM sleep, 826

in pacemaker cells, 873 Calcium deficiency (plant), 636-637 Calcium phosphate, 843 California condor, 1040 California sea otter, 1039 Calmodulin, 289, 839 Calories, 26, 887-888 *Calumorys phicander*, 594 Calvin, Melvin, 147 Calvin-Benson cycle

energy requirements, 146

isotope-labeling experiments, 146-147

linkage of photosynthesis and respiration, 151-152

overview of, 137, 138, 147, 248

products of, 147-148 Calyx, 522, 666 CAM. See Crassulacean acid metabolism

Camarhynchus, 416 Cambrian period, 382

evolutionary change in, 387

fauna of, 392-393

major events in, 380-381 Camels, 693 Camembert cheese, 536 cAMP See Cyclic AMP Campanula, 433

cAMP phosphodiesterase, 290 cAMP receptor protein. See CRP

protein cAMP second messenger systems, 290, 291 Canadian lynx, 979 Canary grass, 653 Canavanine, 683 Cancellous bone, 844-845 Cancer cells, 155 Cancer Genome Anatomy Project, 350 Cancers

caused by viruses, 343

cellular characteristics, 155

characteristics of, 342-343

gene amplification and, 275

genetic diagnosis and screening, 346
 genetic mutations and, 343-344
 human genome sequencing and, 350
 inhibition of cell death and, 304
 lymph nodes and, 877
 oncogenes in, 344, 345
 pathway of events in, 345-346
 prevalence of, 342
 protein degradation and, 277
 Ras protein and, 286
 targeting with monoclonal antibodies, 364
 telomerase and, 264
 tumor suppressor genes in, 344-345 Cancer treatment
 gene therapy, 347-348
 metabolic inhibitors, 347 *Candida albicans*, 533 Canine teeth, 894, 895 *Canis familiaris*, 167 Cannel coal, 512 Cannibalism, 334 Capacitance vessels, 878 Capacitation, 754 Cape buffaloes, 983 Capillaries, 875
 autoregulatory mechanisms and, 882
 blood pressure and flow in, 876
 defined, 868
 peritubular, 915, 916, 927, 920
 pulmonary, 857, 859
 water movement and material exchange in, 876-877
 See also Air capillaries *Capsicum anuum*, 974 Capsid, 241 Capsules
 bacterial, 59
 mosses, 506-507
 nontracheophytes, 504 Captive propagation, 1039-1040 Carbohydrases, 896 Carbohydrates
 biochemical roles of, 43
 categories of, 43
 energy yields from, 887
 general formula, 43
 interactions with other macro-molecules, 53
 membrane-associated, 82
 modified by functional groups, 46
 monosaccharides, 43-44
 polysaccharides, 45-46
 storage molecule for, 888 Carbon
 covalent bonding, 21-22
 as nutrient, 634
 optical isomer, 32 Carbon-14, 381 Carbon cycle, 1001-1003 Carbon dioxide

atmospheric, 1000
 in autoregulation of the circulatory system, 882
 carbon cycle, 1001-1003
 effects on breathing rate, 864
 global warming and, 1003
 and photorespiration, 149
 in photosynthesis, 136, 137, 147, 248, 150, 151
 physical properties of gas exchange, 849, 851-852
 transport in blood, 862-863, 876 Carbon dioxide fixation
 Calvin-Benson cycle, 147, 248
 in C 4 plants, 150
 in crassulacean acid metabolism, 151
 and photorespiration, 149 Carbonic acid, 862, S63 Carbonic anhydrase, 862-863,
 876 Carboniferous period, 500, 508
 climate of, 383
 evolutionary trends in, 388
 major events in, 3S0-3S1
 origin of coal and, 512
 plant evolution in, 518 Carbon monoxide
 in brown algae bladders, 489
 hemoglobin and, 860
 as neurotransmitter, 789 Carbon skeletons
 in anabolic and catabolic inter-conversions, 131-132
 in nutrition, 889 Carbonyl group, 31 Carboxyhemoglobin, 862 Carboxyl group, 31, 37-38 Carboxylic acids, 31
 Carboxypeptidase, 105, 901 Carcinogens, 344 Carcinomas, 343 Cardiac cycle, 872 Cardiac muscle, 697, 872-6"-
 contraction in, 834-S3?
 Frank-Starling law and, 879 *Cardinalis cardinalis*. 592 Cardinals, 592
 Cardiovascular disease, 878-879 Cardiovascular system, 866. See also Circulatory systems Caribou, 594. 967 Carnivores,
 995, 996
 feeding adaptations, 893-894
 teeth, 895 Carnivorous plants, 634, 644 *Carollia perspicillata*. 594 fJ-Carotene, 51-52
 absorption spectrum, 240
 plant biotechnology and, 327 Carotenoids, 51-52, 141 Carotid arteries, 883 Carotid bodies, 864, 884 Carp, 267 Carp bones,
 843 Carpels, 521, 666, 672
 evolution of, 522, 523 Carrier proteins, 88 Carrying capacity, 964
 effects of disturbances on, 966
 human population growth and, 972
 population management and, 970, 971 Cartilage, 696, 697, 843
 in cartilaginous fishes, 584
 tracheal, 857, 858 Cartilage bone, 844 Cartilaginous fishes, 584-585,

696, 843, 917 Cartilaginous skeleton, 843 Casparian strips, 623 Caspase, 303 Cassowaries, 447, 448 Castor beans, 670 Castor canadensis, 887 Castration, 741

Catabolic pathways

integration with anabolic paths, 132

in tercon versions, 131 Catabolic reactions, 96 Catabolite repression, 254 Catalysis, 36

Catalysts, 102. See also Enzymes *fi*-Catenin, 756, 761, 762-763 Catfish, 812

Catlmranthus roseus, 1032 Cations, 24

primary active transport, 89

in soils, 638-639, 640 Cats, 189, 935 Cattle egrets, 983 Cauliflower, 399 Cave ecosystems/habitats, 998,

2034 CD28, 361 cDNA. See Complementary

DXA cDNA libraries, 319 CD4 protein, 366, 374 CD8 protein, 366 Cecum, 902, 903 ced genes, 302-303 Cell adhesion, 82-83, 83

recognition proteins, 83-84 Cell body, of neurons, 775 Cell communication

gap junctions, 84, 85, 292

plasmodesmata, 62, 76, 292-293

See also Cell signaling Cell cycle

checkpoints, 159

cyclin and cyclin-dependent kinase signals, 158-159

growth factors, 159

phases of, 158

tumor suppressor genes and, 34? Cell death, 155, 173-174 Cell dedifferentiation, 299 Cell determination

defined, 295

distinguished from differentiation, 296

embryonic induction and, 301-302

polarity and, 300-301

primary embryonic organizer and, 762, 763 Cell differentiation

defined, 295

differential gene expression in, 296-300

distinguished from determination, 296

reversible and irreversible, 296-299

transcription factors and, 271 Cell division

essential processes in, 155

in eukaryotes, 157

important consequences of, 256

interphase, 157-158

nuclear envelope in, 64

with polyploidy, 173

in prokaryotes, 155-157

See also Cell cycle; Cytokinesis;

Meiosis; Mitosis Cell fate, 295 Cell junctions

desmosomes, 84, 85 gap junctions, 84, 85 tight, 84-85

See also Plasmodesmata Cell movements amoeboid, 833 by cilia and flagella, 831-833 in gastrulation, 760 microtubules and microfilaments in, 833 See also Motility Cellobiose, 44 Cell reproduction. See Cell

division Cells, 8

cell theory, 55 cytoskeleton, 72-76 dedifferentiation, 299 developmental potential, 295 diffusion within, 85 emergence and evolution of, 3,

476-480 endosymbiosis arid, 70, 479 eukaryotic, 58, 59-63 extracellular structures, 76-77 genome constancy, 299-300 organelles of, 63-72 osmosis, 86-87 plasma membrane, 58 prokaryotic, 58-59 surface area-to-volume ratio,

55-56 viewing with microscopes,

56-57 See also Animal cells; Plant

cells Cell signaling

effects of, 290-292 essential components of,

281-282 in plants, 647 receptors, 282-285 signal types and sources,

279-281 transducers, 285-290 Cell theory. 55 Cellular immune response in immune disorders, 372 major histocompatibility complex proteins, 366-367 overview of, 359, 364-365 phases of, 367, 368-369 self-tolerance, 367-368 T cell receptors, 365-366 Cellular respiration, 68 citric acid cycle, 227, 118,

122-125 energy yields, 130 overview of, 115, 117 in plants, 151-152 pyruvate oxidation, 117, 118,

122, 223 respiratory chain, 227, 118,

125-129 using nitrate reduction, 129 Cellular slime molds, 497-498

Cellulases, 466, 902 Cellulose

biological functions, 45

digestion in herbivores, 902-903

microfibrils, 656-657

in plant cell walls, 76

structure, 45 Cell walls

of brown algae, 489

in cell evolution, 478-479

of diatoms, 487-488

microtubules and, 74

in prokaryotes, 58-59, 463-464

of protists, 481

See also Plant cell walls Cenozoic era, 380-381, 390 Center for Disease Control, 886 Centipedes, 571 Central dogma, 220-221 Central nervous system. 774-""

autonomic nervous system and, 822, 823

components of, 814

development of, 816

information flow and, 815

limbic system, 818-819

nuclei, 818

processing visual information, 823-824

reticular system, 818

spinal cord anatomy and functioning, 817-818

See also Brain Central sulcus, 820 Centrioles, 60, 75-76, 161

in early embryo formation, 756

sperm contributions to zygotes, 754 Centromeres, 160, 167, 260 Centrioles, 160-161, 262 Century plants, 968, 969 Cephalization, 546 Cephalochordata, 578, 583 Cephalopods (Cephalopoda), 560, 562, 578

chromatophores and, 847

eye of, 807-808

hydrostatic skeleton and propulsion in, 841-842 Ceratocystis idnii, 1037 Cerebellum, 816, 818 Cerebral cortex

characteristics of, 819

frontal lobe, 820

language areas, 828-829

occipital lobe, 821

parietal lobe, 820 . sleep, 825-826

temporal lobe, 819 Cerebral hemispheres, 816, 819

language areas, 828-829 Cerebrum, 816

evolutionary increase in size, 821

regions of, 819-821

sleep, 825-826 Cereus giganteus, 959 Certhidea olivacea, 416 Cervical cancer, 277 Cervical caps, 746

42 iii labor um chloride, 206 2

cGM] l lic-GMP Chaetognatha, 565-566, 574, 578 Chagas disease, 483 Cham terminators. See Stop

codons Chambered nautilus, 391 Chaparral, 1022 Chaperone proteins, 285 Chaperonins, 42,231,690 Chora, 501,502 Characters, 178,428

continuous, 189

discontinuous, 189 Chargaff, Erwin, 203 Chargaff's rule, 203, 220 Charophytes, 501-502, 503 Cheeses, eukaryotes and, 536 Chelicerates (Chelicerata), 569,

574, 578 Chelonia, 589-590 Chelonia mydas, 590, 738 Chemical bonds

covalent, 20-23

defined, 20

electron behavior in, 19-20

ester linkages, 473

ether linkages, 473

glycosidic linkages, 44, 45

ionic, 24

nonpolar interactions, 24-25

peptide linkages, 37-38, 229

phosphodiester linkages, 47⁸, 204, 214

types of, 22 Chemical equilibrium, 100, 104 Chemically-gated channels, 778 Chemical reactions, 19-20, 25-26

activation energy barrier, 102-103

catalysts, 102

endergonic and exergonic, 99

energy uptake and release in, 99-100

enzymes, 102-106

equilibrium, 100, 104

free energy, 100

and membranes, 92, 93

reversible, 29

spontaneous and nonspontaneous, 99

temperature and, 700

transition-state species, 103

See also Enzyme-catalyzed reactions Chemical signals, 712

cellular, 280-281

pheromones, 534, 713, 797, 847, 935

See also Cell signaling;

Hormones; Signal transduction pathways Chemical synapses, neuromuscular junction, 785-786 Chemical weathering, 638

Chemiosmosis

evolutionary history, 134

oxidative phosphorylation,

127-129 photophosphorylation,

145-146 Chemoautotrophs, 464-465 Chemoheterotrophs, 464, 465 Chemoreceptors, 797-799

in regulation of breathing, 864 Chemosensors, 795 Chemosensory systems, 797-799 Chemosynthesizers, 635 Chemotherapy, 259, 347 Cherries, 525 Cherry trees, 667 Chestnut blight, 536, 1037 Chiasmata, 168, 170, 171,

191-192 Chichlosoma bartoni, 996 Chickens

cleavage in, 757

extraembryonic membranes, 767-767

gastrulation in, 763-764 Chilies, 974 Chimpanzees, 596, 597

diversity of behavior in, 944

relationship and similarity to humans, 6, 431 Chitin, 46, 529, 567, 842 Chitons, 560, 562, 562 Chlamydia, 471, 748-749

Chlamydia

C. psittaci, 471

C. trachomatis, 256, 749 Chloranthaceae, 525 Chlorella, 147, 492 Chloride, 24

membrane potential and, 777, 779 Chloride transporters, 334 Chlorine

in animal nutrition, 890

in plant nutrition, 636 Chloromycetin, 230 Chlorophylls

absorption spectrum, 240

in antenna systems, 142

molecular structure, 242

nutrient deficiencies and, 636

in photoautotrophic bacteria, 464

in photosystems I and II, 243, 144, 145

as reducing agent, 142-143
 types of, 141 Chlorophytes (Chlorophyta), 477, 503
 diversity in, 492
 endosymbiosis and, 495, 496
 life cycles, 492-494 Chloroplast DNA (cpDNA), 431 Chloroplasts, 62, 67
 chemiosmosis in, 145-146
 endosymbiosis and, 70, 479, 494-^95
 in Euglena, 483
 function of, 63
 genetic code and, 224
 light reactions of photosynthesis, 142-146
 structure and function, 68-69
 Chlorosis, 636 Choanocytes, 547 Choanoflagellida, 477, 494 Cholecystokinin, 729, 903 Cholera, 467 Cholesterol
 corticosteroids and, 725
 elimination of, 901
 familial hypercholesterolemia, 333-334, 335
 lipoproteins and, 904, 905
 in membranes, 80-81
 receptor-mediated uptake, 91
 sources of, 52 Chondrichthyes, 578, 584 Chondrocytes, 696 Chondrus crispus, 491 Chordates (Chordata), 435
 hemichordates and, 581-582
 nervous system, 774
 shared characteristics, 582
 subgroups and number of living species, 578
 tunicates, 582-583 Chordin, 309 chordin gene, 543 Chordomesoderm, 765 Chorion, 767, 768 Chorionic gonadotropin (hCG),
 769-770 Chorionic villus sampling, 768,
 769 Chorus frogs, 980, 982 Chory, Joanne, 660-661 Chromatids, 160
 chiasmata, 270, 171
 crossing over, 270,171
 meiosis, 167, 268-269, 170-171
 mitosis, 262, 163-164 Chromatin, 62, 63, 160
 effects on gene transcription, 273-275
 meiosis prophase, 268,171
 mitosis prophase, 262, 163
 types of, 274 Chromatin remodeling, 274 Chromatophores, 846-847 Chromium, 890 Chromophores, 453 Chromoplasts, 69,
 70 Chromosomal abnormalities, 335
 fragile-X syndrome, 335-336 Chromosomal mutations, 234,
 235-236 Chromosomes, 63
 artificial, 316, 349

autosomes, 194
 daughter, 263, 164
 DNA rearrangements, 275
 DNA replication, 209-212
 in eukaryotes, 159-160, 260
 histones in, 160, 262
 homologous pairs, 165-166
 karyotyping, 167
 linked genes, 190-191
 in meiosis, 167, 168-169, 170-171
 meiotic errors and, 172-173
 in mitosis, 162-163, 163-164
 mutations in, 234, 235-236
 nucleosomes, 160, 262
 physical and genetic map markers, 349
 in prokaryotes, 156
 tetrads or bivalents, 171
 transposable elements and, 249
 See also Sex chromosomes *Chrysaora fuscescens*, 548 *Chrysolaminarin*, 487 *Chthamalus stellatus*, 975, 976 Chylomicrons, 900, 901, 904 Chyme, 899 Chymotrypsin, 902 Chytrids (Chytridiomycota), 530, 531, 533-534 Cicadas, 572 Cichlid fish, 712 Cilia, 480
 ciliates and, 485, 486, 487
 diverse uses of, 831
 in flatworms, 554
 in lungs, 858
 microtubule interactions in, 832-833
 motions of, 832
 in rotifers, 555
 structure and function, 74-75 Ciliary muscles, 808 Ciliates, 477, 485-487 Ciliophora, 477 cl protein, 254, 255 Circadian clock, 937-939 Circadian rhythms
 circadian clock, 937-938, 939
 circadian clock genes, 939
 in distance-and-direction navigation, 942-943
 entrainment, 937
 overview of, 673-674, 937
 photoperiodism and, 674 Circannual rhythms, 939 Circular chromosomes, 210 Circular muscle layer, 897 Circulatory systems
 blood, 879-882
 closed systems, 867-868
 components of, 698, 866

control and regulation of, 882-884
 in distribution of hormones, 713
 in diving mammals, 884
 evolutionary trends in vertebrates, 868-871
 fish countercurrent heat exchanger, 704-705
 gastrovascular cavities, 867
 human heart, 871-875
 in major protostomate phyla, 574
 open systems, 867
 perfusion and, 853
 transport of respiratory gases, 860-863
 vascular system, 875-879

Circumcision, 176, 740 Cirri, 486 Cirripedia, 569 Cisternae, 65 Citellus parryi, 594 Citrate, 223,"
 124
 Citric acid cycle, 217, 118, 122-125
 allosteric regulation, 133
 evolutionary history, 134
 interaction with metabolic pathways in plants, 152 Clades, 435 Cladonia subtenuis, 540 Cladophora, 492 Clams, 561 Classes,
 434 Classification systems
 biological names, 433—134
 evolutionary relationships in, 435-436
 hierarchical, 9-10
 Linnean, 433-434, 435
 purposes of, 435
 significance of, 432-433 Class switching, in B cells, 371 dathrin, 91, 92 Clathrina coriacea, 547 Clavicle, 843 day 638-639 Clean
 Air Act, 1004 Cleavage, 761
 blastomere determination, 758-759
 in deuterostomes and proto-stomes, 545
 effects of yolk on, 757
 in mammals, 757, 758
 mitotic spindles and cleavage planes, 757-758
 overview of, 756
 patterns of, 757 Cleavage furrows, 756 Cleft grafting, 677 Cliff swallows, 405-406 Climate. See Global climate Clitoris, 741,
 742 Cloaca, 737 Clock genes, 939 Clonal anergy, 361 Clonal deletion, 360-361 Clonal selection, 359-360 Clones
 asexual reproduction and, 165
 plant reproduction and, 676
 prokaryotes and, 245 Cloning
 animals, 297-299
 genes, 314-318
 plants, 297 Closed systems, 97, 98-99 Clostridium
 C. botulinum, 467, 471

C. tetani, 467, 471 Clotting factors, 880-881 Clover, 409, 976, 977 Club mosses, 501
 characteristics of, 511-512
 Devonian period, 508
 leaves of, 510 Cnemidophorus uniparens, 734 Cnidarians (Cnidaria), 545, 548, 549-551
 biradial symmetry, 546
 gastrovascular cavity, 867, 894
 hydrostatic skeleton, 841
 nematocysts, 846
 nervous system, 774
 regeneration in, 733
 subgroups and number of living species, 578 Cnidocytes, 548, 549 Coacervates, 453 Coal, 388, 512 Coat color
 gene-environment interaction, 189
 inheritance in rabbits, 186
 in mice, 188 Coated pits, 91, 92 Coated vesicles, 91, 92 Cobalamin, 892, 893 Cobalt, 890 Coccus, 461 Cochlea, 802-803, 804
 Cockleburs, 650, 665, 672 Cockroaches, 895 Coconut seedlings, 670 Codominance, 187 Codons, 223-225 Coelomic fluid, 914,
 915 Coelom, 544, 545, 560 Coelomates, 544, 545 Coenocytes, 490 Coenocytic hyphae, 531 Coenzyme A (CoA), 122, 123
 Coenzymes, 107, 108, 891 Coevolution
 in angiosperms, 523-524
 diffuse, 985
 in plant-herbivore interactions, 681-682
 species-specific, 985-986 Cofactors, 107
 Coffee plantations, 1034, 1035 Cohesins, 164 Cohorts, 962 Coitus interruptus, 746 Cold deserts, 1020 Cold-hardening, 690
 Coleoptera, 574 Coleoptiles, 648
 phototropism in, 653, 654 Coleus, 624 Collagen, 696, 697, 843
 in blood clots, 880
 in extracellular matrix, 77 Collecting duct, 919 Collenchyma, 608, 609, 610, 615 Colon, 897, 901-902 Colon cancers, 345-346
 Colonial protists, 494 Colonies, bacterial, 245 Colonization, island biogeographic model and, 1012 Colony-stimulating factor, 323 Color blindness, 195-196 Color vision, 809 Columbus, Christopher,
 750 Columnar epithelial cells, 695,
 696 Comb jellies, 551-552, 578 Commensalism, 975, 983 Commerson's frogfish, 586 Common beans, 684 Common bile duct,
 899, 900 Common grackles, 423
 Common names, 433 Communication, 935-937 Communities, 8 Community ecology
 amensalisms and commensalisms, 975, 982-983 coevolution, 985-986 competitive interactions,
 976-978 effects of species on community structure, 986-987 indirect interactions, 988-989 mutualisms, 983-985 niches, 975,
 976 parasite-host interactions,
 978-979 predator-prey interactions,
 979-982 resources, 975-976 succession, 987-988 types of interactions in, 974-975 Compact bone, 844, 845 Companion cells,
 521, 610, 631,
 632 Comparative genomics, 256, 262 Competition, 974 effects of, 976-978 introduced competitors, 1Q 3Z. uTseisile
 protostomes,
 574-575 types of, 976, 978 Competitive exclusion, 976 Competitive inhibitors, 109 Competitors, 974 Complementary base
 pairing, 48, 204, 205 DNA replication and, 206, 209 Complementary DNA (cDNA),
 319 Complementary genes, 188 Complement proteins, 356-357 Complement system, 356-357 Complete cleavage, 757

Complete metamorphosis, 572,

716, 717 Complex cells (visual cortex),

823 Complex ions, 24 Complex leaves, 511 Complex tissues (plant), 610 Compound eyes, 807 Compound leaves, 606 Compound umbels, 522 Computational biology, 320 Computer modeling, of spider

webs, 407-^08 Concentration gradients, diffusion and, 85 Concentricycloidea, 578, 581 Concurrent blood flow, 855 Concurrent multiplier system,

920-921 Condensation reactions, 35 Conditional mutants, 234 Conditioned reflexes, 827 Condoms, 746, 750 Conduction, in thermoregulation, 702, 703 Conduction deafness, 804

Cone cells, 809, 810. See also

Photoreceptors Cones (plant), 518, 520 Confocal microscopy, 57 Confuciusornis sanctus, 591 Conidia, 533, 536-537 Conifers (Coniferophyta), 501, 517, 518, 519

cones, 518

life cycle, 519-520

seeds, 519-521 Coniosporium, 988 Conjugation

in bacteria, 245-246, 247

F factors and, 249

in paramecia, 487 Connective tissues

in bone development, 844

overview of, 696-697

in skeletal systems, 843

stomach, 695, 697, 698 Connexin, 292 Connexons, 84, 85, 292, 789 Connochaetes, 957 Conotoxin, 847 Consciousness, 829 Consensus sequences, 269 Consensus trees, 430 Conservation biology

causes of endangerment and extinction, 1033-1039

Ecoregion-Based

Conservation, 1042

establishing parks and reserves, 1040, 1042

estimating current rates of extinction, 1030-1032

market issues and, 1043

overview of, 1030

preservation of biodiversity, 1043

preventing species extinctions, 1039-1040

proportion of U.S. species extinct or at risk, 1034

restoring degraded ecosystems, 1042-1043

significance of species extinctions, 1032-1033 Conservative replication, 206 Conspecifics, 947 Constant region, of

immunoglobulins, 362, 363, 369, 371 Constipation, 902 Constitutive enzymes, 250 Contagious diseases, death rates

from, 970 Continental climates, 994 Continental drift, 382, 390, 1008 Continuous characters, 189 Contraception

barrier methods, 746

coitus interruptus, 746

controlling male fertility, 747

preventing implantation, 747

preventing ovulation, 746

rhythm method, 745
 sterilization, 747 Contractile vacuoles, 72, 480, 481 Contragestational pill, 747
 Contralateral neglected syndrome,
 820-821 Controlled systems 698-699 Convection, in thermoregulation,
 702-703 Convergent evolution, 427 Cook Strait, 1010 cooperative behavior. 953 Copepods (Copepoda), 569 Copper
 in animal nutrition, 890
 in plant nutrition, 636 Copper mines, plants and, 689 Coprophagy, 903 Copulation, 7 Coral grouper, 586 Coral roots, 551
 gradients in species richness, 1027
 red algae and, 491 Corals, 550-551
 chemosensation in, 797
 regeneration in, 733 Corens, Karl, 178 Corepressors, 252-253 Cork, 617, 679
 Cork cambium, 613, 615, 617 Corms, 676 Corn
 abscisic acid-deficient mutants, 660
 adaptations to dryness, 686
 domestication, 1028
 hybrid vigor in, 188-189
 seed anatomy 670 Cornea, 302, 808 Corn earworm moth, 679 Cornflowers, 522 Corn oil, 50 Cornusflorida, 527 Corolla, 522,
 666 Corona, of rotifers, 555 Coronary arteries, 879 Coronary infarction, 879 Coronary thrombosis, 879 Corpora cardiaca, 716
 Corporal allata, 716 Corpus callosum, 828 Corpus luteum, 744, 745 Corridors, 1035-1036 Cortex (kidney), 919 Cortex
 (plant), 614 Cortical granules, 754, 755 Corticosteroids, 725-726 Corticosterone, 725 Cortisol, 52
 effects of, 726
 half-life, 729-730
 receptor for, 285
 structure of, 725 Corylus cornuta, 667 Corynebacterium
 C. diphtheriae, 467
 C. parvum, 462 Cost-benefit analyses, in behavioral ecology, 948 Co-stimulatory signals, 361 Cotyledons, 525, 648, 670
 Countercurrent blood flow, 854,
 855 Countercurrent heat exchanger, 704-705
 Courtship displays
 hybridization experiments, 927
 in mallards, 926-927
 See also Mating behavior Covalent bonds
 formation of, 20-22
 multiple, 22-23
 orientation of, 22
 phosphodiester linkages, 47-48
 unequal sharing of electrons, 23 Cowbirds, 395 Cows, foregut fermentation in,
 445, 446, 466 cpDNA. See Chloroplast DNA C₃ plants, 149, 150-151 C₄ plants, 149, 150-151
 leaf anatomy, 618
 plasmodesmata, 293 Crab grass, 150 Crabs, 797 Cranial nerves, 816 Cranium, 843 Crassulaceae, 151 Crassulacean acid
 metabolism

(CAM), 151, 628, 689 Crayfish, 570, 796 Creation science, 13-14 Crenarchaeota, 473 Cretaceous period
evolutionary radiation of birds, 591
evolutionary trends in, 390, 393
major events in, 380-381
mass extinction during, 384, 391-392 Cretaceous-Tertiary boundary,
384 Cretinism, 722 Crews, David, 734 Crick, Francis, 202, 203, 204, 220 Crickets, 572, 932-933 Crinoidea, 578, 580-581
Crinoids, 388
Cristae, in mitochondria, 68 Critical day length, 671 Critical periods, 929, 930 Crocodilians (Crocodylia), 436, 590
circulatory system, 869-870 Cro-Magnons, 598, 599 Crop milk, 714 Cro protein, 254, 255 Crops (digestive organ), 895
Crossbreeding experiments,
933-934 Crossing over, 268
during bacterial conjugation, 246, 247
in homologous chromosomes, 170, 171, 191-192
between linked genes, 191-192 Crossopterygii, 587 Cross-pollination, 777 Crotalus molossus, 812 Crown gall, 469 CRP-cAMP
complex, 253-254 CRP protein, 253-254 Crustaceans (Crustacea), 569-571, 574, 578
Crustose lichens, 540, 541
Cryo electron microscopy, 57
Cryptochromes, 662
Crystallization (bird songs), 932
Csrl-1 gene, 407
Ctenes, 551
Ctenophores (Ctenophora), 545,
546, 551-552, 578 Ctenucid moths, 982 CTLA4 protein, 367, 372 Cuboidal epithelial cells, 695, 696 Cuckoos, 395 Cud, 902
Culex pipens, 167 Culture
defined, 925
human, 599, 944 Cumulus, 753-754 Cup fungi, 536 Cuticle (animal)
in ecdysozoans, 564-565, 565
of exoskeletons, 842 Cuticle (plant), 502, 611, 618 Cuttlefish, 847 Cyanide, 409 Cyanobacteria, 4, 59
creation of atmospheric oxygen and, 454-455, 466
endosymbiosis and, 495, 496
general characteristics of, 470
in lichens, 540
motility in, 462
in nitrogen fixation, 640
photosynthesis in, 146, 464
stromalites and, 454, 455 Cycads (Cycadophyta), 501, 517,
518, 519 Cycas, 519 Cyclic AMP (cAMP)
in CRP-cAMP complexes, 254
functions of, 49, 714
regulation of, 290

as a second messenger, 287

slime molds and, 498 Cyclic electron flow, 143-144 Cyclic GMP (cGMP), 289

in phototransduction, 806, 807 Cyclin-dependent kinase (Cdk),
158-159 Cyclins

cell cycle signaling, 158-159

centromere duplication, 160 Cyclosporin, 368 *Cynanthus latirostris*, 887 Cypress trees, 650, 687 *Cyprinus carpio*, 167
Cysteine

disulfide bridges, 36

genetic code for, 224

plant sulfur metabolism and, 643

side chain, 37 Cystic fibrosis, 334, 341 Cytochrome c, 125, 126, 127

evolutionary change in amino acid sequences, 441, 442-443 Cytochrome oxidase, 125, 126,
227 Cytochrome P450s, 907 Cytochrome reductase, 225, 126, 227

Cytokines, 368

in B cell class switching, 371

humoral immune response and, 367

overview of, 355

phagocytes and, 358 Cytokinesis, 155, 158

plant and animal cells, 164, 265

prokaryotes, 157 Cytokinins, 647, 658 Cytoplasm

in amoeboid movement, 833

in prokaryotes, 58

targeting polypeptides to, 231, 232 Cytoplasmic determinants, 301 Cytoplasmic dynein, 164 Cytoplasmic inheritance, 196
Cytoplasmic receptors, 284-285 Cytoplasmic segregation,
300-301 Cytoplasmic streaming, 73, 497 Cytosine, 47, 48

in DNA, 203, 204, 205

DNA point mutations and, 338

methylation, 275

tautomeric form, 236, 237 Cytoskeleton

in animal cells, 60

in cell evolution, 479

functions of, 62, 72

intermediate filaments, 72, 74

microfilaments in, 72-73

microtubules in, 72, 74-76, 833

in plant cells, 62 Cytosol, in prokaryotes, 58 Cytotoxic T (T_c) cells, 366, 367, 368

Dactylaria, 532

Dactylella, 532

Daffodils, 327

DAG. See Diacylglycerol

Daily torpor, 710

Daisies, 667

Daltons, 17

Dam projects, aftereffects of, 991

Damselflies, 572, 951

Danaus plexippus, 967

Dances, of honeybees, 936-937

Dandruff, 695

Dark reactions. See Calvin-Benson cycle

Darwin, Charles, 414, 438-139 plant studies of, 646, 653 theory of evolution, 2, 379, 395, 396-397, 426

Darwin, Francis, 653

Darwinian theory, 396-397

Darwin's finches, 962

Date palms, 527

Daucus carota, 522

Daughter chromosomes, 263, 164

DAX1 gene, 194

Day length, flowering and, 671-672

Daylilies, 527

Day-neutral plants, 672

DDT, 907, 1040

Deafness, 804

Deamination, 236, 237

Death rates

density-dependent and density-independent, 965-966

in population dynamics, 962 Decalopda, 569 Decapods, 569 Deciduous forests

temperate, 1018

tropical, 1024 Declarative memory, 828 Decomposers, 995, 996

in ecosystems energy flow, 997-998

See also Saprobies Decomposition, as secondary

succession, 988 Deep-sea ecosystems, 998 Defecation, 895 Defense systems

of animal prey, 980, 981, 982

See also Animal defense systems; Immune system; Plant defenses Deforestation rates, 1031 Dehydration, 693 Dehydration reactions, 35 Deletions, 235, 236, 335 Delivery, 770, 771 Demography, 962 Denaturation, 42 Dendrites, 775 Dendritic cells, 355, 373 Dendroica, 435

D. kirtlandia, 1039 Dendrotoxin, 847 Denitrification, 466, 642-643 Denitrifiers, 642, 643 Dense connective tissue, 696

Density-dependent factors,

965-966 Density-independent factors,

965-966 Density labeling, 206, 207-208 Dentine, 894, 895 Dentrifiers, 466

Deoxyribonucleic acid. See DNA Deoxyribonucleoside triphosphates, 209, 214, 215 Deoxyribonucleotides, 48 Deoxyribose, 43, 44, 47, 220 Depolarization, 779, 780 DepoProvera, 746 Deprivation experiments, 927 Derived traits, 427

constructing a simple phy-logeny with, 428-430

distinguishing from ancestral traits, 428 Dermal tissue system (plant), 611 Dermaptera, 572 Dermophus mexicanus, 588 Desert plants, 685-687 Deserts

cold, 1020

hot, 1021

nurse plants, 959

spatial distribution of plants, 960, 961 Desmazierella, 988 Desmodus rotundus, 910 Desmosomes, 74, 84, 85

Desmotubules, 292, 293 Detergents, as surfactants, 858 Determinate cleavage, 545 Determinate growth, 612, 613,

671 Determination. See Cell determination detl mutant, 660 Detoxification, biological, 907 Detritivores, 995, 996, 997-998.

See also Saprobes Deuteromycetes

(Deuteromycota), 530, 539 Deuterostomes chordates, 581-583 developmental characteristics,

545 echinoderms, 579-581 evolutionary trends, 577 phylogeny of, 579 shared, derived traits, 552 subgroups and number of living species, 578 Development, 5-6

body segmentation, 305-308

defined, 294

embryonic induction, 301-302,

303 evolutionary perspectives,

308-309 mosaic and regulative, 759 pattern formation, 302-305 polarity and, 300-301 processes of, 294-296 See also Animal development; Plant development Devonian period

evolutionary trends in,

387-388 major events in, 380-381 plant evolution in, 508, 510 Diabetes mellitus, 372

insulin production in biotechnology, 325 type I, 723-724 type II, 729 Diacylglycerol (DAG), 288 Dialysis, 324

Diaphragm (anatomical), 858-859, 863, 897 Diaphragms (contraceptive), 746 Diarrhea, 902 Diastole, 872, 873 Diatomaceous earth, 488 Diatoms, 477, 487-488 2,4-Dichlorophenoxyacetic acid

(2,4-D), 656 Dichotomous branching, 509 Dicots. See Eudicots Dictyostelium discoideum, 498 Dideoxyribonucleoside triphosphates, 214, 215 Diencephalon, 816 Diener, Theodore, 244 Diesel, Rudolf, 51 Dietary fiber, cholesterol elimination and, 901 Differential gene expression in cell differentiation, 296-300 in establishing body segmentation, 305-308

globin gene family, 268 transcription factors and, 300 See also Development; Gene regulation; Transcriptional regulation Differential interference-contrast

microscopy, 57 Differentiation. See Cell differentiation Diffugia, 481 Diffuse coevolution, 985 Diffusion

across membranes, 85-86 within cells and tissues, 85 facilitated, 87-88, 89 Fick's law, 852 of gases, 849-850, 851-852 physical nature of, 85-86 simple, 86, 89 See also Osmosis Digestibility-reducing compounds, 982 Digestion

autonomic nervous system

and, 822 control and regulation of, 903 digestive enzymes, 896 in gastrovascular cavities, 894 lysozyme evolution and, 445⁴⁶ in mouth and stomach,

898-899 prokaryote-aided, 466 tubular guts, 894-896 Digestive enzymes, 896, 897 bile, 899-900 in lysosomes, 66-67 of mouth and stomach, 898,

899 pancreas and, 900 sources and functions of, 902 Digestive tract

absorption of nutrients,

900-901 absorption of water and ions,

901-902 absorptive and postabsorptive

periods, 903-904 autonomic nervous system

and, 822, 834 control and regulation of, 903 digestion in, 898-900 in major protostomate phyla,

574 organs in and functions of, 698 peristalsis in, 896-897, 897-898 smooth muscle contraction,

834 tissue layers, 896-897 Dihybrid crosses, 182, 184-185 Dihydroxyacetone phosphate

(DAP), 119, 122 Dikaryons, 533, 537 Dimethyl sulfide, 1004 Dinoflagellates, 477, 484, 551 Dinosaurs, 389, 590-591 Dioecious flowers, 522 Dioecious organisms, 192, 736 Diomedea melanophris, 941 Dionaea muscipula, 634 Dioscorea, 432-433

Dipeptidase, 902

2,3 Diphosphoglyceric acid (DPG), 862

Diphtheria, 360, 467

Dipln/Ilobotlumi latum, 554

Diploblastic animals, 545

Diploid cells, 166

Diplontic life cycle, 166, 493

Diptera, 574

Directional selection, 405, 406

Direct transduction, 286-287

Disaccharides, 43, 44

Discontinuous characters, 189

Discrete characters, 189

Diseases

bacterial, 466[^]167 death rates from, 970 Koch's postulate, 467 multifactorial, 335 See also Genetic diseases

Disk flowers, 522

Disparity. See Visual disparity

Dispersal

distribution of species and,

1009 in marine systems, 1026-1027 in response to environmental changes, 966

Dispersive replication, 206

Displays

in communication, 935-937 See also Courtship displays

Disruptive selection, 405, 406

Distance-and-direction navigation, 941-943

Disulfide bridges, 36, 39, 40

Diving reflex, 884

DNA base sequences

analyzing in molecular evolution studies, 440 as evolutionary trait, 431

DNA-binding proteins, 273

DNA chips, 321-322, 350

DNA (deoxyribonucleic acid) antiparallel orientation of

strands, 48 benzpyrene and, 199 central dogma and, 220-221 Chargaff's rule, 203 chemical composition, 203 chip

technology, 321-322 cleaving and rejoining,

311-314 compared to RNA, 47, 220 complementary base pairing,

48, 204, 205, 206, 209 deoxyribose in, 43, 47 double helix, 48, 203, 204, 205 eukaryotic, 159-160, 260-261 evolutionary relationships

and, 49 evolution of, 454 5' and 3' ends, 204, 205, 208 identified as the genetic material, 199-202 informational content, 48 in kinetoplasts, 483 methylation, 274-275 nitrogenous bases in, 47, 48 noncoding, 446-447 nucleic acid hybridization, 268

nucleotide components, 4~

point mutations and. pot) merase chain reaction,

214. 216-217 positional cloning. 336-337 in prokaryotes, 156-157, 4e>2 proofreading and repair,

212-214 rearrangements within chromosomes, 275 recombinant, 313-314 repetitive sequences, 263-265 replication, 206-212 restriction endonucleases, 312 sequencing, 214, 275 sticky ends, 314 structure, 202-205 synthetic, 319

s\ nthetic mutations, 319-320 telomeres, 263-264 template strand, 222 transcription, 222-223 triplet repeats, 339 X-ray crystallography, 202-203 D\ A fingerprinting, 328-329 DNA helicase, 209, 222 DNA libraries, 318-319 DNA ligase, 209, 212, 314 DNA polymerases, 206, 315 in DNA proofreading and

repair, 212, 213 in DNA replication, 208, 209,

210, 211 mutations caused by, 236 in PCR technique, 216-217 polymerase I, 211, 212 polymerase III, 208, 111, 212 DNA probes, 313 DNA replication

cell division and, 155 lagging strand, 211-212 leading strand, 211 mechanisms in, 208, 209-212 Meselson-Stahl experiment,

207-208 possible modes of, 206 in prokaryotes, 156 DNA sequencing, 214, 215, 254.

See also Genomics DNA technology antisense RNA, 322 applied to plant tissue culture,

677 complementary DNA, 319 DNA chips, 321-322 DNA fingerprinting, 328-329 DNA insertion techniques,

316-317 DNA splicing, 313-314 gel electrophoresis, 312-313 gene cloning, 314-318 gene libraries, 318-319 gene therapy, 347-348 genetic markers, 315, 317-318 homologous recombination,

320-321 knockout experiments, 320,

321 medically useful products

from, 323-325 nitrogen fixation and, 641-642 overview of, 311-312

positional cloning, 336-337

power of gene amplification in,

qualities of transgene hosts, 315

restriction endonucleases, 312

restriction fragment length polymorphisms, 337

synthetic DNA, 319

synthetic DNA mutations, 319-320

vectors, 315-316

See also Biotechnology DNA testing, 341, 342 DNA topoisomerases, 164, 210 DNA transposons, 265 DNA viruses, 241 Docking proteins, 231 Dodder, 643 Dogs

chromosome pairs, 267

olfaction in, 794, 798

territory marking, 935 Dolly (cloned sheep), 298 Dolphins, 594 Domains

in biological classification, 9, 10, 459-461

of proteins, 266 Domestication, 600, 1028 Dominant traits

dihybrid crosses, 182, 283

incomplete dominance, 186-187
 monohybrid crosses, 278, 179-180
 pedigree analysis, 284, 185
 test crosses, 181-182 Donkeys, 267 Dopamine, 789 Dormancy, in seeds, 647, 649-650, 652, 670 Dorsal horn (spinal cord), 817 Dorsal lip of the blastopore, 761,
 762-763, 765 Dorsal-ventral axis, determination of, 309, 756 Dorsal-ventral patterning, genes
 involved in, 766-767 Dose-response curves, 729 Double bonds, 23 Double fertilization, 521, 524,
 668, 669 doublesex (dsx) gene, 934 Doublets, microtubular, 75 Douching, 746 Douglas firs, 620 Doves, 714 Downregulation,
 729 Down syndrome, 173, 442 DPG. See 2,3 Diphosphoglyceric
 acid Dpp, 309 Dragonflies, 572
 nymphs, 980, 982 Dreaming, 825, 826 Drosera, 644 Drosophila
 behavioral genetics studies, 933, 934
 bithorax mutation, 445
 body segmentation, 305-308
 cleavage in, 757 D. balioptera, 420
 D. conspicua, 420
 D. silvestris, 420
 D. subobscura, 403⁰⁴
 eyeless mutation, 309
 genetic similarity in sympatric species, 420
 genetic studies with, 190
 genome, 262
 homeotic mutations in, 307-308
 maternal effect genes, 305-306
 ommatidia, 807
 sex determination, 194
 sex-linked inheritance, 195
 speciation in, 415
 trisomy in, 442 Drought stress, 273 Dryas, 987-988 Dryopteris intermedia, 514 Dubautia menziesii, 422 Duchenne's
 muscular dystrophy,
 334,335, 336-337 Duckbilled platypus, 593, 798 Ducks, courtship displays,
 926-927, 927 Dugesia, 554 Dulbecco, Renato, 348 Dulse, 492
 Duodenum, 897, 899, 903 Duplications, 235, 236 Dutch elm, 536 Dutch elm disease, 1037 Dwarfism, pituitary, 719-720
 Dynein, 75, 832, 833 Dysticus marginalis, 573
 Eagles, DDT and, 907 Ear, 802-803 inner, 801-803 middle, 802, 803, 804 See also Auditory systems Ear drum, 802, 803
 Ear ossicles, 802, 803, 804 Ear pinnae, 802, 803 Earth
 biogeochemical cycles,
 1001-1005 biogeographic regions, 1011 carrying capacity for human
 growth, 972 conditions at the origin of life,
 451⁵² early history of, 450 geological history, 379,
 380-384 major ecosystem compartments, 999-1001 temperature regulation by the atmosphere, 1000 Earthworms, 558,

559 circulatory system, 868 digestive system, 895, 896 hermaphroditism in, 736 hydrostatic skeleton and locomotion in, 841, 842 metanephridia, 914, 925 nervous system, 774 Earwigs, 572 East Coast fever, 483 E-Cdk2 protein, 160

Ecdysone, 716, 717 Ecdysozoans, 553

arthropods and relatives,

567-574 characteristics of, 564 with cuticles, 564-567 general characteristics, 574 phylogeny, 565

subgroups and number of living species, 578 Echinids, 593 Echinacea purpurea, 522 Echiniscus springer, 568 Echinoderms (Echinodermata) biradial symmetry, 546 evolutionary innovations in,

579 lineages of, 580-581 regeneration in, 733 subgroups and number of living species, 578 Echinoidea, 578, 581 Echolocation, 812 Ecton burchelli, 955 Ecological biogeography, 1008,

1011-914 Ecological communities, 974 Ecological niches, 975, 976 Ecological succession, 987-988 Ecology

biogeography and, 1011-1014

study of, 947

types of interactions in,

974-975 See also Behavioral ecology; Community ecology; Population ecology; Restoration ecology Ecoregion-Based Conservation

(ERBC), 1042 Ecoregions, 1042 EcoRI, 312, 313, 337 Ecosystem compartments,

999-1001 Ecosystems

aftereffects of damming rivers,

991 agricultural, 998-999 biogeochemical cycles,

1001-1005 chemosynthetically powered,

998 defined, 991 energy flow in, 994-999 global climate, 991-994 major compartments,

999-1001 net primary production,

994-994 restoring degraded systems,

1042-1043 value to humanity, 1032-1033 Ectocarpus, 489, 490 Ectoderm, 545

extraembryonic membranes

and, 768 fate of, 759

formation of, 759, 760, 761 in neurulation, 765, 766 Ectomycorrhizae, 540 Ectopic pregnancy, 758 Ectoprocta, 555, 556, 578

Ectotherms

behavioral thermoregulation, 702

blood flow and heat exchange, 702-703, 704

compared to endotherms, 701-702

countercurrent heat exchanger, 704-705

defined, 701

heat production in, 704 Edema, 877, 878 Edge effects, 1035 Ediacaran fauna, 386, 387 EEC See Electroencephalogram Effector cells, 360, 363 Effector proteins, 284 Effectors, 110, 774

chromatophores, 846-847

cilia, 831-832

defined, 831

electric organs, 847

glands, 847

in jumping, 831

microfilaments, 833

microtubules, 832-833

muscle contraction, 833-841

nematocysts, 548, 549, 846

in regulatory systems, 698 Efferent blood vessels, 854

arterioles, 916, 920 Efferent nerves, 815 Egg costs, breeding behavior and, 950 Eggs

of amniotes, 588, 589

blocks to polyspermy, 754, 755

contributions to zygote, 754-755

cytoplasmic determinants, 301

cytoplasmic rearrangements, 755-756

egg-sperm recognition mechanisms, 753-754

fertilization in humans, 741-742

mechanisms of fertilization, 753

nuclear transplant experiments, 297

polarity in, 300-301

yolk, 756

See also Ova; Shelled egg EIN2, 659, 660 Ejaculation, 741, 745 EKGs. See Electrocardiograms Elastic connective tissue, 696 Elastin, 696 Elaters, 505 Elderly, death rates from disease, 970 Electrical fields, detection of, 812 Electrical synapses, 789 Electric catfish, 847 Electric eel, 847 Electric fishes, 812, 847, 937 Electric organs, 847 Electric signals, 712, 937 Electrocardiograms (EKGs), 874, 875 Electrochemical gradients, 622

Electrodes, measuring membrane potential with, 777 Electroencephalogram (EEG), 825-826 Electromagnetic spectrum, 139 Electromyogram (EMG), 825 Electron carriers

in glucose metabolism, 116, 117, 118

See also Nicotinamide adenine dinucleotide; Flavin adenine dinucleotide Electronegativity, 23 Electron flow, in photosynthesis, 143-145 Electron microscopes, 56, 57 Electrons

charge, 18

in chemical bonding, 19-23

mass, 17

orbitals, 20

redox reactions, 115-116

respiratory chain, 125-126, 127 Electron shells, 20, 21 Electrophoresis, 225, 312-313 Electroporation, 317 Electoreceptors, 812 Electrosensors, 795 Elementary bodies, 472 Elements

atomic mass, 19

atomic number, 19

defined, 17

mass number, 19

See also Atoms Elephantiasis, 567 Elephants

life cycle, 266

overexploitation of, 1038

thermoregulation in, 703 Elephant seals, 403 Eleutherozoans, 580, 581 Elk, 682

Ellipsoid joint, 845 Elongation factors, 229 Embolisms, 879 Embolus, 879 Embryo development, 294

axes determination, 756

body segmentation, 305-308, 765-767

cleavage, 756-759

extraembryonic membranes, 767-768

gastrulation, 759-765

human, 769-770

induction in, 301-302, 303

neurulation, 765-767

polarity and, 300-301 Embryonic induction, 301-302,

303 Embryonic stem cells, 294, 299 Embryophytes, 500 Embryo sacs, 666, 667 Emergent properties, 9 Emission, 741

Emlen, Stephen, 942 Emus, 447, 448 Enamel, 894, 895 Endangered species

captive propagation, 1039-1040

geographic distribution of

U.S. species, 2042 recovery plans, 1039 See also Conservation biology; Species extinctions Endangered Species Act, 1039

Endemic species, 422, 1009-1010 conservation efforts and, 1040,

1042 of Madagascar, 1040, 2042 Endergonic reactions, 99,

101-102 Endocrine glands, 713, 714-715 Endocrine system

adrenal gland, 724-726 atrial natriuretic hormone,

729, 727-728, 925 control of digestion, 903 control of insect molting, 716 control of kidney function,

922, 923-924 control of male sexual function, 741 control of ovarian and uterine

cycles, 743-745 negative feedback loops,

721-722 overview of, 698, 714, 715,

728-729 pancreas, 723-724 parathyroids, 723, 724 pineal gland, 727 pituitary gland, 717-722 regulation of blood pressure,

882, 883, 884 thyroid gland, 722-723 See also Hormones Endocuticle, 842 Endocytosis

in capillary exchange, 877 microfilaments in, 833 receptor-mediated, 91, 92 types of, 90-91 Endoderm, 545 fate of, 759

formation of, 759, 760, 762 yolk sac and, 767-768 Endodermis, 614, 623 Endomembrane system, 64-67 Endometrium, 742, 743, 744, 745,

768 Endomycorrhizae, 540 Endoplasmic reticulum in animal cells, 60 dynamic nature of membranes

in, 92 function of, 63 in plant cells, 62 in plasmodesmata, 607 structure and function, 64-65 targeting polypeptides to, 231,

232, 233 vesicles from, 66 Endorphins, 718, 720, 790 Endoscopy, 747 Endoskeletons

bone development and structure, 844-845 bone remodeling, 843-844 in bony fishes, 585 in cartilaginous fishes, 584

connective tissues in, 843

joints, 845-846

in mammals, 593

origins of, 579

in radiolarians, 496
 in vertebrates, 583 Endosperm, 521
 double fertilization and, 668, 669 Endospores, 471 Endosymbiosis
 in cell evolution, 70, 479
 dinoflagellates and, 484
 gut bacteria, 895-896, 902, 903
 multiple events in, 494-495
 in protists, 481
 transposons and, 265 Endotherms
 basal metabolic rate, 705, 706
 behavioral thermoregulation, 702
 blood flow and heat exchange, 702-703
 brown fat, 706
 compared to ectotherms, 701-702
 defined, 701
 shivering, 706
 strategies for decreasing heat loss, 706-707
 thermoneutral zone, 705-706 Endotoxins, 467 End-product inhibition, 110 Endymion nonscriptus, 433 Energetic costs, 948
 Energy
 from chemical reactions, 25-26
 defined, 95
 food calories, 887-888
 transfer in redox reactions, 116
 types of, 96
 See also Free energy Energy compounds
 polysaccharides, 46
 triglycerides, 51 Energy conversion
 and changes in matter, 96-97
 in chemical reactions, 99-100
 laws of thermodynamics, 97-99
 See also Metabolism Energy flows
 chemosynthetically powered systems, 998
 laws of thermodynamics, 994
 net primary productivity, 994-995
 through trophic levels, 995-998 Energy pathways. See Glucose
 metabolism Energy pyramids, 997 Enhancers, 261, 271, 272, 323 Enkephalins, 718, 719, 720, 790 Enlemur fidvus, 1041
 Enolase, 222 Enterococcus, 461 Enterogastrone, 729 Enterokinase, 900, 902 Enthalpy, 97, 98 Entrainment, 674, 937
 Entropy, 97-98 Eucleated eggs, 297
 Enveloped viru
 I n\ ironment(s) carrj ing capacity 964 defined bj ecologists, 94 " influence on e\ olution in animal societies, 957-958 influence

on speciation, 421 internal and external, 694 osmolality and, 911

Environmental adaptation, 4, 7

Environmental toxicology,

Enzyme-catalyzed reactions

chemical equilibrium, 104 enzyme-substrate interactions, 105

reaction rates, 105-106, 110

reduction of activation energy barrier, 102-103, 104-105

substrate concentration, 105-106

turnover number, 106 Enzyme inhibitors, 106-107,

108-109 Enzyme regulation

allosteric, 110, 222, 132-134

transcriptional, 250-254 Enzymes

activation energy barrier and, 102-103, 104-105

active sites, 104, 105, 106

allosteric, 109-110, 222

coenzymes, 107, 108

cofactors, 107

constitutive, 250

defined, 36, 102

enzyme-substrate interactions, 105

genetic diseases of, 332

induced fit, 106-107

inducible, 250

kinases, 119

names of, 104

one gene, one enzyme hypothesis, 218-220

pH and, 111, 222

prosthetic groups, 207, 108

reaction rates, 105-106, 110

regulation of, 108-109, 222

signal transduction and, 291

specificity, 103-104, 106

and temperature, 112 Enzyme-substrate complex, 104,

105, 106-107 Eosinophils, 355, 357 Ephedra, 521 Ephemeroptera, 572 Epiblast, 763, 764, 768 Epicotyl, 648 Epicuticle, 842
Epidermal growth factor (EGF),

714 Epidermis, 611

of leaves, 618

in plant defenses, 679

in roots, 613-614

of stems, 615 Epididymis, 739, 740 Epiglottis, 897, 897-898 epsy, 827 i >by, 761

Epinephrine, 280, 287, 713, 725

glycogen metabolism and, 291

half-life, 729

receptor for, 284

regulation of blood pressure and, 883

targets and actions of, 729, 728 Epiphanes senta, 555 Epiphytes, 1025

Episomes, 248. Set' also Plasmids Epistasis, 188

gene-for-gene resistance in plants, 681 Epistylis, 486 Epithelial cells, 695, 696 Epithelial tissues

desmosomes, 85

overview of, 695, 696

stomach, 695, 697

tight junctions, 84-85 Epstein-Barr virus, 343, 373 Equatorial plate

meiosis, 269

mitosis, 263, 164 Equilibrium

in chemical reactions, 100, 104

in solutions, 85 Equilibrium organs, 801-803 Equisetum, 512 Equus, 167 era mutation, 628 Erection, 740 Erie, Lake, 1005

Error signals, 698, 699 Erysiphe, 536 Erythrocytes, 879-880. See also

Red blood cells Erythromycin, 230 Erythropoietin, 159, 880, 881

biotechnology and, 323, 324-325 Escherichia coli, 256, 462, 469, 1005

cell division in, 156

circular chromosome in, 256

in DNA technology, 315, 317

generation time, 464

genome, 260, 261, 262

gram staining, 463

growing in the laboratory, 245

in intestines, 902

regulation of lactose metabolism in, 250

signal transduction in, 281-282

transposable elements and, 249 Esophageal sphincter, 898 Esophagus, 897, 898 Essential amino acids, 889, 890 Essential elements (plant)

deficiency symptoms, 635-636

defined, 635

identification of, 637

macronutrients, 635, 636

micronutrients, 635, 636 Essential fatty acids, 891 Ester linkages, 473 Esters, 50 Estradiol, 725, 726

Estrogens, 52, 283, 726

oral contraceptives and, 746 in ovarian and uterine cycles,

743, 744, 745 in pregnancy and labor,

769-770 targets and actions of, 729

Estrus, 743

Ether linkages, 473

Ethology, 926

Ethyl alcohol, 129, 230

Ethylene, 280 activities of, 647 chemical structure, 658 effects on stems, 659 in fruit ripening, 659 leaf abscission and, 658 signal transduction pathway, 659-660

Etiolation, 662

Etoposide, 347

ETR1, 659, 660

Euascomycetes, 535, 536-538

Eubalaena australis, 887

Eucalyptus regnans, 620

Eucalyptus trees, 686, 893

Euchromatin, 274

Eudicots (Eudicotyledonae), 434, 526, 527, 604 embryo development in, 669 leaf anatomy, 617-618 root anatomy, 624 secondary growth, 615-617 seedling apical hooks, 659 shoot development patterns,

648 Stoma and guard cells, 627 tissue systems in, 622 vascular bundles, 625

Euglena, 483

Euglenoids (Euglenozoa), 477, 483, 495, 496

Eukaryotes (Eukarya), 9, 20, 60, 61, 459, 460, 461 atmospheric oxygen and, 382 cell cycle in, 158 characteristics of, 5 chromosomes in, 159-160 cilia and flagella, 74-75 compartmentalization in,

62-63 division in, 157 emergence of, 4 endosymbiosis and, 70,

494¹⁹⁵, 749 evolutionary relationships,

467-468 evolution of, 476-180 expression vectors for, 323 location of glucose metabolism in, 117, 228 organelles in, 62-72 overview of, 59, 62 transcriptional gene regulation

in, 270-276 transcription in, 223, 272, 274 as transgene hosts, 315 translation in, 225-231

Eukaryotic genome

characteristics of, 259-261 fruit fly, 262 nematode, 262

repetitive sequences, 263-265 sequencing, 261, 263 structure of protein-coding

genes, 265-268 transcription, 266 yeast, 261, 262

Eunice, 732

Euplectella aspergillum, 547

Euplotes, 486

Europa, 27

European robins, 927

European starlings, 941, 942

Euryarchaeota, 473—474

Eusociality, 954-955

Eustachian tube, 802, 803

Eutherians, 595, 739

Eutrophication, 470, 1004-1005

Evaporation in heat loss, 707 physical properties, 27-28 in thermoregulation, 702, 703

Everest, Mount, 849, 851

Evergreen forests boreal, 1017 tropical, 1025

Evolution

adaptation and, 395, 396 and allosteric regulation,

133-134 altruism and, 954-956 of animal defense systems, 372 of animal societies, 956-958 associative mating, 404 atmospheric oxygen and, 380,

382, 454-455, 466 constraints on, 410-411 Darwinian theory, 396-397 defined, 379-380 developmental perspectives,

308-309 effect of humans on, 393 energy conversion and, 4 gene flow, 402 genetic code and, 224 genetic variation and, 408—409 germ-line mutations, 402 homeostatic mechanisms and,

5 long-term vs. short-term, 411 major events and trends, 3-7,

386-390 major faunas, 392-393 of metabolic pathways, 134 methods of studying, 406-408 mutation and, 236-237 natural selection, 2-3, 404-406 origins of life, 3, 450—454 phenotypic plasticity and, 410 population genetics and,

398-402 random genetic drift, 402—404 rates of change and extinction,

390-392 See also Molecular evolution

Evolutionary classifications, 431-432 DNA studies and, 48 See also Phylogeny; Phylogenetic trees

Evolutionary developmental biology, 308-309

Evolutionary innovations, 392 Evolutionary radiations, 386, 422-423 of birds, 591 of echinoderms, 579 Evolutionary reversals, 427 Evolutionary theory Darwinian, 396-397 development of, 1-3 natural selection, 2-3, 404-406 Excision DNA repair, 214 Excitatory postsynaptic potential

(EPSP), 787, 788 Excitatory synapses, 786-787 Excited state, 139-140, 142 Excretory systems

adaptations for water conservation and salt excretion, 917-918 of invertebrates, 914-915 kidney control and regulation,

922-924 kidney structure and function,

918-921 mechanism used by, 911 nephron structure and function, 915-916, 917 nitrogen excretion, 912-914 organs in and functions of, 698, 911 Exercise, cardiac effects, 879 Exergonic reactions, 99 activation energy barrier,

102-103 ATP cycling and, 101-102 Exhalation, 858, 859, 863. See also

Tidal breathing Exocrine glands, 715 Exocytosis, 91-92 Exons, 265, 266, 267, 268, 269 Exoskeletons

of arthropods, 567 cuticles, 564-565, 566 molting and, 564, 715 Exotoxins, 467 Expanded-tip tactile receptors,

799 Expansins, 657 Experimentation, 12-13 Expiratory reserve volume, 856,

857 Exploitative competition, 978 Exponential growth, in populations, 963-965 Expression vectors, 322-323 Expressivity, 189 Extensor muscles, 817-818, 845 External fertilization, 736-737 External gills, 853 External intercostal muscles,

858-859 Extinction rates, 391-392 estimating, 1030-1032 island biogeographic model and, 1012 Extinctions. See Extinction rates;

Species extinctions Extracellular matrix, 76-77, 696 Extracellular structures, 76-77 Extraembryonic membranes, 764^765

formation of, 767-768 prenatal testing and, 768, 769

Extreme halophiles, 474

Eye

accommodation, 808 anatomy of, 808 compound, 807 development, 301-302, 309 gap junctions in lens cells, 292 independent evolution, 807 retina structure and function, 808-812

Eye-blink reflex, 827

Eye cups, 807

eyeless gene, 309

Face recognition, 819, 820 Facilitated diffusion, 87-88, 89 Factor VIII, 323 Facultative anaerobes, 464 Facultative parasites, 532 Falcons, 907, 1040 Falco peregrinus, 1040 Fallopian tube, 741 Familial hypercholesterolemia (FIT), 333-334, 335, 347 Families, 434

parenting behavior and, 956 Far-red light, 661-662, 673 Fas receptor, 367 Fast block to polyspermy, 754 Fast-twitch muscle fibers,

840-841 Fat digestion, 899-900 Fate maps, of blastulas, 758 Fats

digestion and absorption of, 899-900, 901

energy storage, 132, 888

energy yields from, 887

overview of, 49-51 Fat-soluble vitamins, 891-892 Fatty acids

absorption in small intestine, 901

anabolic interconversion, 131

catabolic interconversions, 131

essential, 891

saturated and unsaturated, 50

structure of, 49-50

synthesis for storage, 133 Fatty tissue. See Adipose tissue Faunas

Ediacaran, 386, 387

major evolutionary groups, 392-393 "Feather duster" worms, 559 Feathers, 592, 707 Feather stars, 580, 581 Feces, 895, 902

coprophagy, 903 Feedback

in regulation of breathing, 864

in regulation of hormone receptors, 729

in regulatory systems, 698-699

See also Negative feedback Feedforward information, 699 Feeding adaptations

carnivores, 893-894

herbivores, 893

teeth, 894, 895 Feeding behaviors, 927-928 Females

hormonal control of sexual behavior, 931-932

mating behavior and, 950, 951, 952

parenting behavior, 956

sex organs, 741-742 Femur, 843

Fenestrations (capillary), 876, 877 Fermentation

energy yields, 129, 130

evolutionary history, 134

glycolysis and, 120

overview of, 115, 129, 130

Pasteur's study of, 114 Fernald, Russell, 712 Ferns, 501

Devonian period, 508

leaves of, 513

life cycle, 266, 513-515

polyploidy in, 417 Ferredoxin, 144-145 Ferritin, 277

Ferrocactus acanthodes, 428 Fertilization

blocks to polyspermy, 754, 755

double, in angiosperms, 521, 524

egg and sperm contributions to the zygote, 754-755

egg cytoplasmic rearrangements, 755-756

egg-sperm recognition mechanisms, 753-754

external, 736-737

in humans, 741-742

internal, 737

mechanisms of, 753

pronuclei in, 339

and sexual reproduction, 165, 166 Fertilization cone, 753, 754, 755 Fertilization envelope, 754, 755 Fertilizers, 639

manufacture of, 641-642 Fetal hemoglobin, 268, 861, 862 Fetus

defined, 726

development of, 769-770 Fevers, 709 F factors, 249

Fibers (sclerenchyma), 608, 609 Fibers (xylem element), 521 Fibrin, 95, 881 Fibrinogen, 697, 881 Fibrous root systems, 605 Fibula, 843

Fick's law of diffusion, 852 Fiddleheads, 513 Fight-or-flight response, 713, 821

Cortisol and, 726

epinephrine in, 725 Figs, 525

Filaments (floral), 521, 522, 666 Filaments (prokaryote associations), 461, 472 Filariasis, 567 Filopodia, 760 Filter feeders, 893

Filtration

in capillaries, 876

in excretory systems, 911, 914 Fins, 584

jointed, 587 Fin whales, 973 Fireflies, 935-936 Fires

plants and, 516

seed germination and, 649

See also Forest fires Fireweed, 649 Firmicutes, 471-472 First polar body, 735, 736 Fischer, Edmond, 287 Fischer, Emil, 106 Fishes

acclimatization and metabolic compensation in, 700, 720

adaptations for water conservation and salt excretion, 917

body plan, 583

bony, 585-586, 587, 917

cartilaginous, 584-585, 696, 843, 917

commercial management of populations, 970

countercurrent heat exchanger, 704-705
 detection of electrical fields, 812
 evolutionary developments in, 583-585
 evolution in, 410, 411
 gas exchange in, 850-851, 852
 internal gills, 852, 853-854, 855
 jawless, 583, 584, 585
 lateral line sensory system, 801
 parenting behavior, 956
 prolactin in, 714
 schools, 586
 speciation in, 417
 taste buds, 798 Fishing industry, mercury pollution and, 991 Fission, 155-157, 462 First filial generation (F₁), 179 Fitness
 inclusive, 954
 in population genetics, 398 Fitzroy, Robert, 396 5' ends, of DNA, 204, 205, 208 Flagella
 bacteria, 59
 chytrids, 534
 diversity of uses, 832
 euglenoids, 483
 microtubule interactions in, 832-833
 motions of, 832
 prokaryotes, 75(n), 462-463
 protists, 480
 stramenopiles, 487
 structure and function, 74-75 Flagellin, 75(n), 462 Flame cells, 914 Flatworms, 553-554
 excretory system, 914
 gas exchange and, 850
 gastrovascular cavity in, 867
 nervous system, 774 subgroups Mid number of li\
 iii; spec ies 578 \ isual system Flavin adenine dinucleotide
 A \1M ;/7 US. 124 Flavonoids, 641, 683 Fleas
 Hexon muscles, 817-818, 845 Flies, 574 chemoreceptor hairs, 797 \ isual s) stem, 807 Flightless buds, 592 e\ olution in, 447,
 448 human-caused extinctions, 1030 Flocking, 953 Floral meristem identity genes,
 671 Floral meristems, 304, 671 Florida scrub jays, 957 Floridean starch, 491 Florigen, 674-675 Flounder, 410, 411 Flowering
 gene expression in, 671 overview of, 648, 649 photoperiodic control of,
 671-675 transition to, 670-671 vernalization and, 675-676 Flowering dogwood, 527 Flowering hormone, 648, 649,
 674-675 Flowering plants, 501
 characteristics of, 521, 603-604 coevolution with animals,
 523-524 double fertilization, 521, 524,

668, 669 evolutionary relationships,
 526 flower structure and evolution, 521-523 fruits, 525
 life cycle, 524-525, 647-649 monoecious and dioecious,
 192 monophyletic groups, 526,
 527, 604 oldest living lineage, 525, 526 origins of, 526 self-compatibility, 432 tissues and tissue systems,
 610-611 vegetative development,
 611-617 vegetative organs, 603-606 Flowers
 associative mating, 404 coevolution and, 523-524 evolution in, 522-523 organ identity genes and,
 304-305 organs and structure of,
 521-522, 666 whorls, 304 Fluid feeders, 893 Fluid mosaic model, 79-82 Fluid tissue. See Blood Flukes, 553, 554
 Fluorescence, 142
 1 luorescence microscopy, 57 1 luorine, 890
 5-Fluorouracil, 347 FMR1 gene, 339 Foliar sprays, 639 Folic acid, 892
 neural tube defects and, 766 Foliose lichens, 540, 542 Follicle cells (ovarian), 753-754 Follicles (hair), 296, 800 Follicles
 (ovarian), 742, 743-744,
 745 Follicle-stimulating hormone (FSH), 718, 719
 male sexual function and, 741
 in ovarian and uterine cycles, 743, 744, 745
 in puberty, 726, 727 Follicular large-cell lymphoma,
 304 Foiling, Asbjorn, 331 Food
 calorie yields, 887, 888
 in protostome evolution, 574
 toxic compounds and, 906-908 Food chains, 996 Food intake, regulation of, 906 Food production. See Agriculture Food
 vacuoles, 71-72
 in protists, 480, 481 Food webs, 996
 energy transfer in, 997-998 Foolish seedling disease, 651 Foot, molluscan, 560 Foraging theory, 949-950 Foraminiferans,
 481, 496 Forbs, 986 Forebrain, 816
 limbic system, 818-819
 reticular system and, 818 Foregut fermentation, 445-446 Forelimbs, evolution in mammals, 3 Forensics, DNA fingerprinting,
 328 Foreskin, 739, 740 Forest biomes
 boreal forests, 1017
 temperate deciduous forests, 1018
 thorn forests, 1023
 tropical deciduous forests, 1024
 tropical evergreen forests, 1025 Forest communities
 amensalisms in, 983
 destruction and fragmentation of, 1034, 1036
 introduced pests, 1037
 pyramids of energy and bio-mass, 997 Forest fires, 516, 966 Forest reserves, 1042 Forestry, 1034, 1036 Fossil fuels
 acid precipitation and, 1004

consequences of burning, 1002-1003

formation of, 1002

peat, 500 Fossils, 380

formation of, 384

using to determine evolutionary pathways, 384-385, 432 Founder effects, 403-404 Founder events, 415 Fovea, 808-809 Fragile-X syndrome, 335-336,

339 Frame-shift mutations, 235 Frank-Starling law, 879 Fraternal twins, 743 Free energy

ATP cycling, 101

chemical equilibrium, 100

citric acid cycle, 122, 124

glucose metabolism, 114—115

glycolysis, 119, 120, 124

in thermodynamics, 97, 98-99 Free radicals, 236 Free-running circadian rhythms,

937 Freeze-fracture technique, 81 Frequency-dependent selection,

409 Frequency (light), 139 Fresh water fishes, speciation in,

417 Freshwater systems

cycling of materials, 999-1000

hydrological cycle, 1001

numbers of species in, 1026 Frogs, 587

blastula fate map, 758

chromosome pairs, 167

egg cytoplasmic rearrangements, 756

evolution and, 411, 427

eye development, 301-302

gastrulation, 761-762

gray crescent, 755

life cycle, 588

mating behavior, 737, 738

neurulation, 765

nitrogenous waste excretion, 914

nuclear transplant experiments, 297

thyroxine hormone in, 714

toxins from, 847 Fronds, 513 Frontal lobe, 819, 820

Broca's area, 828, 829 Fructose, 44 Fructose 1,6-bisphosphate (FBP),

220 Fruit fly. See *Drosophila* Fruiting structures

of basidiomycetes, 538, 539

of cup fungi, 536

in fungi, 530, 533 fruitless (*fru*) gene, 934 Fruits

auxin and, 656

diffuse coevolution, 985

ripening, 659

in seed dispersal, 670

types of, 525

variety in, 670 Fruticose lichens, 540, 542 Fucoxanthin, 488-489

Fuel metabolism, control and regulation of, 903-905 Fugu parda, 907 Fulcrums, 845 Fumarate, 223, 124 Fumaroles, 1004 Functional genomics, 255-256 Functional groups

with carbohydrates, 46

described, 31-32

in macromolecules, 35 Fundamental niches, 975, 976 Fungal diseases, 533 Fungi, 9-10

ascomycetes, 535-538

asexual reproduction, 265

associations with other organisms, 539-542

basidiomycetes, 538, 539

chytrids, 533-534

defined, 529

deuteromycetes, 530, 539

distinct from protists, 529-530

general biology of, 529-533

gigantic, 529

life cycle, 266

as normal flora, 356

phyla of, 530, 533

phylogeny, 533

in pine litter decomposition, 988

reproduction in, 532-533

zygomycetes, 534—535 Funk, Casimir, 892 Fur, in reducing heat loss, 707 Furbish's lousewort, 1031-1032 Furchgott, Robert, 289 Furcifer revocosus, 1041 Fusarium moniliforme, 539 Fusicoccum, 988 Fusin, 374

Fynbos vegetation, 2022, 1032-1033

Gage, Phineas, 814 Galactosamine, 46 Galactose, 44 fi-Galactosidase, 250 P-Galactoside permease, 250 Galago senegalensis, 595 Galapagos finches, 415-4Y7 Galapagos Islands, 396 Galapagos tortoises, 1037 Gallbladder, 897, 899, 900, 903 Gambusia, 996 Gametangia, 493, 502

chytrids, 534

nontracheophytes, 504 Gamete intrafallopian transfer

(GIFT), 749 Gametes, 165

emergence of, 5

formation of, 734-736 Gametic isolation, 419 Gametogenesis

oogenesis, 735, 736

overview of, 734-735

spermatogenesis, 735-736 Gametophytes, 490

conifers, 520
 ferns, 514-515
 flowering plants, 521, 524, 666-667
 in homosporous and heterosporous, 522
 nonseed tracheophytes, 507
 nontracheophytes, 504
 plant life cycle, 501
 seed plants, 516-517

Gamma-aminobutyric acid (GABA), 787, 789, 790
 Gamma radiation, 19
 Ganglion cells, retinal, 810-812
 Ganglion/Ganglia, 774

in annelids, 558
 of autonomic nervous system, 821, 822, 823
 defined, 821
 Gap genes, 306, 307
 Gaping (bird behavior), 423
 Gap junctions, 292, 789

in cardiac muscle, 872
 described, 84, 85
 Garner, W. W., 671
 Garrod, Archibald, 220, 331
 Gas exchange

Fick's law, 852
 physical properties of, 849-852
 Gas exchange systems, 698

bird lungs, 854-856
 defined, 853
 fish gills, 853-854, 855
 insect tracheae, 853
 tidal breathing, 856-857

See also Respiratory gas exchange
 Gasterosteus aculeatus, 391
 Gastric glands, 898, 899
 Gastric ulcers, 898-899
 Gastrin, 903

Gastropods (Gastropoda), 560,
 562, 578
 Gastrovascular cavity, 549, 867,
 894
 Gastrulation

in amphibians, 761-762
 in birds and reptiles, 763-764
 extraembryonic membranes, 767-768
 in mammals, 764-765
 overview of, 759
 primary embryonic organizer, 762-763

in sea urchins, 759-761
 Gas vesicles, 462
 Gated ion channels, 283

in facilitated diffusion, 87-88
 See also Voltage-gated channels

Gatun Lake, 1027
 food web, 996
 Gazelle thomsoni, 947
 G caps, 269, 277
 Gel electrophoresis, 312-313
 Gemmae, 505

Gene amplification, 275-276
 Gene duplication

creation of new gene families, 442-445
 protein diversification and, 443-444, 445
 types of, 442

Gene expression, 270

blocking with antisense RNA,

322 central dogma, 220-221 coordinating several genes,

272-273 genetic code and, 223-225 one gene, one polypeptide

hypothesis, 218-220 point mutations and, 234-235 posttranscriptional events, 276 posttranslational events,

231-233, 277 primary RNA transcript processing, 268-270 in prokaryotes, 222, 249-254 signal transduction and,

291-292 transcription, 222-223,

270-276 translation, 225-231, 276-277 See also Differential gene

expression; Gene regulation; Transcriptional regulation Gene families defined, 443 in *Drosophila*, 262 in eukaryotes, 267-268 gene duplication and, 442-445 immunoglobulin genes,

368-371 See also Globin gene families Gene flow, 402 Gene-for-gene resistance, 681 Gene guns, 317 Gene homologs

in eye development, 309 medical significance, 262 Gene libraries, 318-319 Gene pool, 398 Generation times

influence on speciation,

421-122 in prokaryotes, 464 Generative cell, 668, 669 Generator potential, 796 Gene regulation combinatorial, 304 posttranscriptional control,

276 in prokaryotes, 249-254 transcriptional control,

270-276 translational and posttranslational control, 276-277 in viruses, 254, 255 See also Differential gene expression;

Transcriptional regulation Genes, 180

amplification of, 275-276

annotation, 262

bacterial conjugation, 245-246,

247 cloning, 314-318 complementary, 188 cytoplasmic inheritance, 196 environmental effects, 189-190 epistasis, 188 genome size and, 446 homologous, 111 1 45

homologous recombination,

320-321 hybrid vigor, 188-189 knockout experiments, 320,

321 lateral transfer, 468 linked, 190-191 loci, 181

mapping, 192, 293 methods of identifying,

336-339 mutations, 233-237 polygenes, 189 protein-coding, structure of,

265-268 recombination, 191-192 on sex chromosomes, inheritance of, 195 stress response elements, 272,

273 twin studies, 189-190 See also Alleles Gene sequencing, 261, 263. See

also Genome sequencing Gene therapy, 347-348 Genetic code, 223-225

point mutations and, 234 Genetic diseases, 331

from abnormal or missing

proteins, 332-334 beta thalassemia, 269-270 genomic imprinting and, 339 identifying mutant genes in,

336-339 multifactorial, 335 patterns of inheritance in,

335-336 point mutations and, 338 reproductive technologies

and, 749-750 screening for, 339-341 treating, 346-348 triplet repeats and, 339 Genetic diversity meiosis and, 172 sexual reproduction and, 165,

734 Genetic maps, 192, 293, 349 Genetic markers, 315, 317-318,

349 Genetic recombination. See

Recombination Genetics

gene interactions, 188-190 heritable character traits, 178 Mendel's experiments, 178-180, 181-182 Mendel's laws, 180-183 pedigrees and pedigree analyses, 184, 185 plant breeding contributions to, 176-177 probability calculations, 183-185 Punnett squares, 180-181 studies with *Drosophila*, 190 using viruses and prokaryotes in, 239-240 Genetic screening, 339-341 public policy issues, 350 Genetic transformation, 200-201

Genetic variation

allele frequencies, 399-400 associative mating and, 404 germ-line mutations and, 402 Hardy-Weinberg equilibrium, 399-400 mechanisms maintaining, 408-409 natural selection and, 404-406 neutral theory of molecular evolution and, 439 in populations, 398-400 random genetic drift and, 402-404

Gene transfer

bacterial conjugation, 245-246, 247 by plasmids, 248 in prokaryotes, 245-247 transduction, 247 transformation, 247 transposable elements, 249

Gene trees, 444

Genital herpes, 748-749

Genitalia, 737

Genital warts, 748-749

Genome constancy, 299-300

Genomes

constancy in somatic cells, 299-300 eukaryotic, 259-263 minimal, 256-257 noncoding DNA and, 446-447 prokaryotic, 255-257, 260, 262 size of, 446 universal gene segments, 256

Genome sequencing, 255-257 hierarchical methods, 348-349 Human Genome Project, 348 ownership issues, 350-351 shotgun methods, 349-350 significance of, 350

Genomic imprinting, 339, 755

Genomics, 321 comparative, 256 functional, 255-256 medical applications, 256 minimal genomes, 256-257 rise of, 255

Genotype frequencies calculating, 399-400 Hardy-Weinberg equilibrium, 400^{+2}

Genotypes, 180

determination of phenotype, 410 expressivity, 189 fitness and, 398 penetrance, 189 in population genetics, 398 sexual reproduction and, 408

Genus/Genera, 10, 433, 434

Geoffroy Saint-Hilaire, E., 309, 543

Geographic speciation. See Allopatric speciation

Geologic history

atmospheric changes, 382 climatic changes, 383 continental drift, 382 major periods in, 380-382

methods of dating rocks

(82 volcano 84

ges Bank 970

4 lb

Germ cells, 734 735 Germination (-49-650,

651

etiolation and, 662

sensith it\ to light, 661 Germ layers

late ofc 759

formation of, 759 Germline mutations, 234, 402 (. lestation, 769

in marsupials, 593

See also Pregnancy Gharials, 590 Giant baobob tree, 2042 Giant clams, 561 Giant petrel, 913 Giant sequoias, 539 Giardia, 477, 479 Gibberellafijikuroi, 539, 651 Gibberellins

activities of, 647

deuteromycetes and, 539

discovery of, 651-652

effects of, 652

in seed germination, 650, 651 Gibbons, 596, 597 Gigantism, 719, 720 Gill arches, 853, 854

in jaw evolution, 585 Gill filaments, 853-854 Gills

of basidiomycetes, 538, 539

external, 850, 853

internal, 852, 853-854, 855

of mollusks, 560 Ginkgo, 502, 518, 529 Ginkgo biloba, 518, 529 Ginkgophyta, 502, 527, 518 Girdling, 629 Gizzards, 895 Glacial moraines, succession on,

987-988 Glands

as effectors, 847

endocrine, 713, 714-715, 728-729

exocrine, 715

gastric, 898, 899

mammary 593

poison, 847

salivary, 715, 897

salt, 688, 912, 923, 917

sweat, 79, 715 Glans penis, 739, 740 Glass sponges, 547 Glial cells, 697, 776, 784 Global climate, 991-992

atmospheric circulations, 992, 993

changes during Earth's history, 383

continental, 994

maritime, 994

ocean influences on, 993-994

solar inputs, 992 Global warming, 1003

species extinctions and, 1038-1039 Globin gene families, 267-268, 299-300 differential expression, 268 gene duplications and,

443^144 transcription of, 266, 272 Globin proteins, 268

beta thalassemia and, 269-270 evolution in, 443[^]144, 445 sickle-cell anemia and, 234, 333, 336, 341, 342 Glomerular filtration rates, 920,

922 Glomeruli, 915, 916, 927 Glomus caledonius, 540 Glossophaea, 985

Glucagon, 280, 718, 724, 904, 905 Glucocorticoids, 729, 725, 726 Glucogenesis, 904 Gluconeogenesis, 131, 132 Glucosamine, 46 Glucose, 155

facilitated diffusion with carrier proteins, 88 forms of, 43 glucogenesis, 904 glycogen metabolism and, 291 in glycolysis, 117,118, 220 glycosidic linkages, 44 nervous system and, 904 osmotic pressure and, 46 produced by photosynthesis, 148 Glucose metabolism

allosteric regulation, 132-134 anabolic interconversions,

131-132 catabolic interconversions, 131 citric acid cycle, 227, 118,

122-125 electron carriers in, 116, 227,

118 energy yields, 130 fermentation, 129, 230 free energy released by,

114-115, 224 glycolysis, 118-121 location in prokaryotes and

eukaryotes, 227, 118 metabolic pathways in,

116-118 pyruvate oxidation, 117, 118,

122, 223 redox reactions, 115-116 respiratory chain, 227, 118, 125-129 Glucose-1-phosphate, 100 Glucose-6-phosphate, 100, 220 Glucose-6-phosphate dehydrogenase, 234 Glucose transporter, 88 Glue lines, 844, 845 Glutamate, 789, 790, 791 Glutamate receptors, in learning and memory, 789-790, 827 Glutamic acid, 37, 224 Glutamine, 37, 101-102, 224 Glyceraldehyde, 44

Glyceraldehyde-3-phosphate

(G3P), 207, 119, 222, 147, 248, 152 Glycerol, 49, 131 Glycine, 149, 787

genetic code for, 224

as neurotransmitter, 789, 790

side chain, 37 Glycogen, 45, 46, 888, 889 Glycogen metabolism, 291 Glycogen phosphorylase, 291 Glycogen synthase, 291 Glycolate, 149 Glycolipids, 53, 82 Glycolysis

allosteric regulation, 133

catabolic interconversions, 131

energy-harvesting reactions in, 119-121

energy-investing reactions in, 118-119

energy yields, 118, 229, 222, 130

evolutionary history, 134

and fermentation, 120, 129

free energy released by, 229, 120, 224

interaction with metabolic pathways in plants, 252

overview of, 115, 116-117,118, 220-222

substrate-level phosphorylation in, 120 Glycoproteins, 53, 64, 82, 83 Glycosides, 683 Glycosidic linkages, 44, 45 Glycosylation, 233 Glyoxysomes, 71 Glyphosate, 327 Gnetophytes (Gnetophyta), 502,

527, 518, 529, 521 Gnetum, 521 Goats

imprinting in, 929

scrapie in, 334

transgenic, 311 Goby, 996

Goiters, 722-723, 893 Golden toad, 587 Golgi, Camillo, 65 Golgi apparatus, 233

in animal cells, 60

dynamic nature of membranes in, 92-94

function of, 63

lysosomes and, 67

in plant cells, 62

structure and function, 65-66 Golgi tendon organs, 800, 801 Gonadotropin-releasing hormone (GnRH), 721, 741

in ovarian and uterine cycles, 743, 744, 745

in puberty, 726-727 Gonadotropins, 743-745 Gonads, 734

hormones of, 729

sex steroids and, 726 Gondwana, 380, 387, 1008, 1011 Gonorrhea, 748-749 Gonothyraca loveni, 548 Gonyaulax, -177, 484

Gooseneck barnacles, 570

Gorilla gorilla, 596

Gorillas, 432, 596, 597

Goshawks, 953

Gout, 913-914

G1 phase, 158

G2 phase, 158

gp41 protein, 373, 374

gpl20 protein, 373

G protein-linked receptors, 284,

291 G proteins, 284

lipid-derived second messengers and, 288

metabotropic receptors and, 788, 789

regulation of, 290 Grackles, 423 Grafting (plant), 677 Grain crops, plant biotechnology

and, 327 Gram, Hans Christian, 463 Gram-negative bacteria, 463,

470-471 Gram-positive bacteria, 463,

471 ^72 Gram staining, 463 Grana, 68, 69 Grand Canyon, 382 Grapes, 652 Grasses, 527

adaptations to dryness, 686

grazing and, 682

interspecific competition, 976, 977

spike inflorescences, 522 Grasshoppers, 194, 572 Grassland communities, 997 Grasslands, temperate, 1019 Gravitropism, 654 Gray crescent, 755, 756, 761, 762 Gray matter, 817 Gray whales, 940 Grazers, 595 Grazing

plant defenses and, 682-685

positive effects on plants, 681-682 Great Barrier Reef, 551 Great egret, 996 Great Lakes, PCBs and, 908 Great Mormon butterfly, 573 Great white sharks, 704-705 Green algae, 477, 501

chlorophytes, 492-494

chloroplasts in, 69

endosymbiosis and, 495, 496

in lichens, 540

origin of plants and, 501-502, 503

symbiosis with sea anemones, 69 Green darner dragonfly, 564 Green fluorescent protein, 218,
 317-318 Greenhouse effect, 1001 Green plant kingdom, 501 Green sea turtle, 590, 738 Grevy's zebra, 2009 Griffith,
 Frederick, 200, 247 Gristle, 843 Gross primary production, 994
 Gross primary productivity, 994 Ground meristem, 612, 614 Ground squirrels, hibernation in,
 710 Ground state, 139-140, 142 Ground tissue system, 611, 615 Groundwater, 1000 Group living, 953 Growth
 in development, 295
 trade-offs with reproduction, 968-969 Growth factors, 714
 cell cycle and, 159
 in embryonic induction, 302 Growth hormone, 323, 718,
 719-720 Growth hormone release-inhibiting hormone, 722 Growth hormone-releasing hormone, 721 GSK-3 protein kinase,
 756 GTP. See Guanine triphosphate GTPases, 290
 Guanacaste National Park, 1043 Guanine, 47, 48
 benzoapyrene and, 236
 in DNA, 203, 204, 205 Guanine triphosphate (GTP)
 citric acid cycle and, 124
 functions of, 49
 G caps and, 269
 G protein-linked receptors and, 284 Guard cells, 611, 618, 627-628,
 660 Guillemin, Roger, 721 Gurdon, John, 297 Gustation, 798-799 Guthrie, Robert, 340 Guts
 tubular, 894-896
 See also Digestive tract Guttation, 624, 625 Gymnophiona, 587 Gymnosperms, 516
 conifer cones, 518
 conifer life cycle, 519-521
 fossil record, 518
 height of, 620
 phyla of, 502, 527, 518 Gypsy moths, 989 Gyrus/Gyri, 819
 angular gyrus, 828, 829
 HAART, 374-375 Haber process, 641 Habitat islands, 965 Habitats, 948
 corridors, 1035-1036
 destruction of, 1034
 edge effects, 1035
 fragmentation of, 1034-1036
 in species preservation, 971 Habitat selection, 948-949 Hadrobunus maculosus, 570 Haemophilus influenzae, 255 Hagfishes,
 583, 584, 737 Hair cells
 auditory, 803, 804
 as mechanoreceptors, 801-802
 Hair follicles, 296, 800 Half-lives, 381
 of hormones, 729-730 Hallucinogens, 982 Halophiles, 474 Halophytes, 688-689 Hamilton, W. D., 955 Hammer, 802, 803
 Hamner, Karl, 672 Hangingflies, 951 Haploid cells, 166 Haplontic life cycle, 166, 493 Harapaphe haydeniana, 571 Harbor seal,
 866 Hardy, Godfrey H., 400 Hardy-Weinberg equilibrium,
 399-400 Hares, 903 Harlequin bugs, 573 Harvestmen, 570 Haustoria, 643 Haversian bone, 845 Haversian systems, 844,

845 Hawaii

Drosophila speciation in, 415

evolutionary radiations in, 422[^]23

human-caused extinctions, 1030, 1038 Hawaiian honeycreepers, 1038 Hawks, fovea of, 808-809 Hazelnut, 667

Hearing. See Auditory systems Hearing loss, 804 Heart

atrial natriuretic hormone, 729, 727-728, 925

blood flow through, 871-872

cardiac cycle, 872

cardiac muscle and heartbeat, 872-874

diving reflex and, 884

electrocardiograms, 875

exercise and, 879

four-chambered, 870-871

Frank-Starling law, 879

hormones of, 729

human, 871-875

kidney function and, 924-925

pacemaker cells, 821, 873, 874

in reptiles, 589, 869-870

three-chambered, 869

tubular, 867

two-chambered, 868-869 Heart attacks

cardiovascular disease, 878, 879

EKGs and, 875 Heart block, 874 Heart murmurs, 872 Heartwood, 617 Heat, solar, 992 Heat detectors, 812 Heat exchange, in thermoregulation, 702-703, 704 Heat of vaporization, 27-28 Heat shock proteins, 42, 690 Heat stress, 272

Heavy chains, of immunoglobulin, 362, 363, 369, 370-371

Heavy metals, plants and, 418,

689 HeLa cells, 155 Helicase, 209, 222 *Helicobacter pylori*, 898-899 Helicoma, 988 *Heliconia rostrata*, 428 *Helicoverpa zea*, 679 Heliozoans, 496 Helix. See Alpha helix *Helix aspersa*, 421 Helix-loop-helix motif, 273 Helix-turn-helix motif, 273, 308 Helper T (T_H) cells, 363, 366, 368

in B cell class switching, 371

HIV and, 373, 374

humoral immune response and, 367 Hematocrit, 879 Heme, 277 *Hemerocallis*, 527 *Hemiascomycetes*, 535-536 Hemichordates (Hemichordata),

578, 581-582 Hemipenes, 738 Hemiptera, 572 Hemizyosity, 195 Hemmings, Sally, 329 Hemoglobin

affinity for carbon monoxide, 860

beta thalassemia and, 269-270

Bohr effect, 862

carbon dioxide and, 862, 863

evolution of, 443-444, 445

fetal, 268, 861
 globin gene families and, 267-268
 oxygen-binding properties, 860-862
 positive cooperativity in, 860
 quaternary structure, 40, 42
 sickle-cell anemia and, 332-333
 translational regulation of, 277 Hemoglobin C disease, 333 Hemoglobin pseudogene, 431 Hemophilia, 176, 196, 334, 335, 347, 880-881 Henricia leviuscula, 580 Hensen's node, 764, 765 Henslow, John, 396 Hepatic duct, 899, 900 Hepatitis B, 343, 748-749 Hepatophyta, 502, 505 Herbicide resistance, 407 Herbicides
 engineering crop resistance to, 327
 selective, 656 Herbivores, 995, 996
 cellulose digestion in, 902-903
 feeding adaptations, 893
 plant defenses and, 682-685
 positive effects on plants, 681-682
 teeth, 895 Hering-Breuer reflex, 864 Heritable traits, 178, 398 Hermaphroditism, 726, 736
 in annelids, 558
 Hermit warblers, 419, 420 Herpes simplex virus, 749 Herring gulls, 927-928 Hershey-Chase experiment, 201-202 Heterochromatin, 274-275 Heterocysts, 470 Heterokaryons, 533 Heteromorphic life cycles, 490, 493 Heteropods, 562 Heterosis, 188-189 Heterosporous, 511, 516-517 Heterotherms, 701 Heterotrophs, 635, 886
 feeding adaptations, 893-894, 895 Heterozygosity
 in Mendelian genetics, 180
 overdominance, 189
 test crosses, 181-182 Hexokinase, 38, 119, 220, 134 Hexoses, 43, 44 Hibernation, 710 Hibernators, overnourishment and, 889 Hiemalora, 552 Hierarchical DNA sequencing, 348-349 High-density lipoproteins (HDLs), 904, 905 Highly active antiretroviral therapy (HAART), 374-375 Highly repetitive sequences, 263 Hindbrain, 816 Hindgut, 895 Hinge joint, 845 Hippocampus, 819
 in learning, 826-827
 in memory, 828 Hirudin, 95, 559 Hirudinea, 558-559, 578 Hirudo, 895-896
 H. medicinalis, 95 Histamine, 713-714
 in inflammations and allergic reactions, 357-358, 372, 876-877
 as neurotransmitter, 790 Histidine, 37, 224, 283 Histones, 160, 262 Historical biogeography, 1008 HIV, 265
 HM.S. Beagle, 396 Hoatzin, 446 Hodgkin, A. L., 783 Holdfasts, 489 Holocene epoch, 390 Holothuroidea, 578, 581 Homeobox, 308, 444 Homeobox genes
 evolutionary significance, 444-445
 evolution of animal bilateral symmetry, 552
 in ray-finned fishes, 577 Homeodomain, 308 Homeostasis, 5, 108, 694-695 Homeotherms, 701 Homeotic genes
 in body segmentation, 766-767

in Drosophila development

evolutionary significance,

444-445 in flowering, 671 gene clusters Homeotic mutations "1)4 Drosophila, 307-308 in loming, 940-941 \ loming pigeons, ^40, 943 Hominids, 597-598 Homo

H. credits, 447-448, 597-598 H. garhi, 597 H. habilis, 597, 598 H. sapiens, 390, 598 (see also Humans) Homologous chromosomes, 165-166 crossing over, 270, 171,

191-192 meiosis, 167, 168-169, 170-171 synapsis, 168, 170 Homologous genes, 111 1 15 Homologous recombination,

320-321 Homologous traits, 427 Homologs, 428 Homoplastic traits, 427 Homoptera, 572 Homospory, 511 Homozygosity

in Mendelian genetics, 180 test crosses, 181-182 Honeybees, 573

behavioral genetic studies,

933-934 dances of, 936-937 heat production, 704 sex determination, 192 Hoofed mammals, feeding ecology and social organization, 957 Horizons (soil), 638 Horizontal cells, 810 Horizontal transmission, 243, 244 Hormone receptors, 728-729 Hormones

adrenal gland, 725-726 behavior and, 931-932 carrier proteins and, 730 cichlid fish and, 712 circulating, 713 control of digestion, 903 control of kidney function,

922, 923-924 control of male sexual function, 741 control of ovarian and uterine

cycles, 743-745 defined, 646 degradation and elimination,

730 dose-response curves, 728 endocrine glands, 713,

714-715 evolution and, 714 half-lives, 729-730 lipid-soluble, 728 local, 713-714 mechanisms of action, 728-730

overview of, 712-713 pancreas, 723-724 parathyroids, 723, 724

pineal gland, 727 pituitary gland, 717-720 regulation of fuel metabolism,

904-905 secondary sex determination,

194 signal transduction pathways

and, 728 synthetic toxins as, 907-908 thyroid gland, 722-723 water-soluble, 728-729 See also Endocrine system; Plant hormones Hormosira banksii, 489 Hornbeam, 667 Hornworts, 502, 502, 503,

505-506 Horsehair worms, 566, 578 Horses

area phylogeny, 2009 chromosome pairs, 267 evolution of, 385, 386 fovea of, 809 human history and, 1028 Horseshoe crabs, 391, 569 Horsetails, 502, 508, 512 Hosts, 974

parasite-host interactions,

978-979 for transgenes, 314, 315 Hot deserts, 1021 Houseflies, 267 Hoverflies, 573 Hox (homeobox) genes

in body segmentation, 766,

767 evolutionary significance, 444--145 H-2 proteins, 366 Hubel, David, 823 Human artificial chromosome

(HAC), 316 Human chorionic gonadotropin

(hCG), 745 Human evolution, 390 australopithecines, 597 bipedalism, 597 brain size and, 598 cerebrum and, 821 hominids, 597-598 language and culture, 598-599 mitochondrial DNA and, 438,

448 molecular phylogenetics and,

448 Neanderthals and, 438 "out of Africa" and "multiple regions" hypotheses, 447^48 Human Genome Diversity

Project, 350 Human Genome Project, 348 Human genome sequencing hierarchical methods, 348-349 Human Genome Project, 348 ownership issues, 350-351 shotgun methods, 349-350 significance of, 350

Human history, biogeography

and, 1028 Human immunodeficiency virus (HIV), 749

infection pathway, 373

proteases and, 233

reproductive cycle, 243, 244, 373-374

transmission, 373, 375

treatments, 374-375 Human insulin. See Insulin Human leukocyte antigens, 366 Human papillomavirus, 749 Humans

abnormal sex chromosome conditions, 194

apoptosis in, 303

causes of death, 970

dehydration and, 693

development of agriculture, 599-600

digestive system, 896, 896-902, 897

Earth's carrying capacity and, 972

effects on evolution, 393

essential amino acids, 889

heart, 871-875

inherited and learned behaviors, 944

intestinal bacteria and, 466

karyotype, 267

major digestive enzymes, 902

nervous system, 774

obesity and overnourishment in, 886, 889, 906

olfaction in, 798

pedigree analyses, 184, 185

phylogeny, 598

population growth, 600, 970-972

pregnancy and birth, 769-771

relationship and genetic similarity to chimpanzees, 6, 432

reproductive system, 739-745

sex determination, 194

sex-linked inheritance, 195-196

sex organ development, 726

sexual behavior, 745-750

skeleton and joints, 843-846

skin pigmentation, 189

survivorship curves, 963

taste buds, 798-799

thermoregulation, 703

totipotency of embryo, 297

twinning in, 759

undernourishment in, 889 Human T cell leukemia virus,

343 Humerus, 843

Hummingbirds, 709-710, 887 Humoral immune response

antibodies, 362, 363-364, 368-371

B cell development into plasma cells, 362-363

monoclonal antibodies, 364, 365

overview of, 359

phases of, 367, 368-369 polyclonal response, 364

Humus, 639-640

hunchback gene, 307, 308

Hunchback protein, 308

Hunt, Sir John, 849

Hunting seasons, 970

Huntington's disease, 184, 339

Huxley, A. F., 783

Hyalophora cecropia, 716, 717

Hybridization experiments, in animal behavior, 927, 932-933

Hybridomas, 364, 365

Hybrids, 419

hybrid vigor, 188-189

Hybrid zone, 419, 420

Hydra, 733

gastrovascular cavity, 867 Hox genes and, 444

Hydrochloric acid, in digestion, 897, 898

Hydrogen, as nutrient, 634

Hydrogen bonds, 22, 23, 27 in complementary base pairing, 47, 48 in DNA, 48, 204, 205, 214 in a helices, 38-39 in (3 pleated sheets, 39 between ribonucleotides, 48

Hydrogen sulfide, 1004

chemosynthesizers and, 635 intestinal bacteria and, 902 in photosynthesis by anaerobic bacteria, 146

Hydroid cells, 506

Hydrological cycle, 1001

Hydrolysis, 35 in digestion, 896

Hydrolytic enzymes, 896

Hydronium ion, 29

Hydrophilic molecules, 24

Hydrophobic interactions, 21, 24-25

Hydroponic culture, 635

Hydrostatic skeletons, 546, 841-842

Hydrothermal vents, 559

Hydroxyl group, 32

Hydrozoans (Hydrozoa), 549-550, 578

Hygienic behavior, 933-934

Hylobates lar, 596

Hymen, 741

Hymenopterans (Hymenoptera), 574 eusociality in, 954-955 parthenogenesis in, 733-734

Hypercholesterolemia, 91

Hyperpolarization, 779, 780

Hypersensitive response (plants), 680, 681

Hypersensitivity (immune system), 372

Hyperthermophiles, 473

Hyperthyroidism, 722-723

Hypertonic environments, fungi and, 531

Hypertonic osmoregulation, 911, 922

Hypertonic solutions, 87

Hyphae, 531

dikaryotic and heterokaryotic, 533 Hypoblast, 763, 764, 768 Hypocotyl, 648 Hypometabolism, 884 Hypothalamus, 724, 816

in food intake regulation, 906 neurohormones of, 721 pituitary gland and, 717,

720-721 in puberty, 726-727 regulation of blood pressure

and, 883 regulation of kidney function,

922 in thermoregulation, 707-709 Hypothermia, 709-710 Hypotheses, 10-12 Hypothetico-deductive method,

11-12 Hypothyroidism, 722, 723 Hypotonic osmoregulation, 911,

912 Hypotonic solutions, 87 Hypoxia, 880 Hypoxia-inducible factor (HIF-

1), 880 Hypsclodoris, 561 H zone, 836, 837

I band, 836, 837

Ice, 26-27

Icelanders, 350-351

I-cell disease, 233

Ice plants, 686

Identical twins, 759

IgA antibodies, 363

IgD antibodies, 363

IgE antibodies, 363

i gene, 252

IgG antibodies, 363-364

IgM antibodies, 363, 370-371

Iiwi, 1038

Ileum, 897, 899, 901

Ilium, 843

Imbibition, 650

Immediate memory, 828

Immune system

cellular immune response, 359, 364-368

characteristics of, 358-359

clonal selection in, 359-360

evolution of, 372

humoral immune response, 359, 362-364

immunological memory, 359, 360

key proteins in, 354-355

natural and artificial immunity, 360

overview of, 353, 698

primary and secondary immune responses, 360

self-tolerance, 359, 360-362, 367-368 Immune system disorders

AIDS and HIV, 373-375

autoimmunity, 372

hypersensitivity, 372 Immunizations, 360

passive, 364

Immunoassays, 364 Immunoglobulin genes, 368-371 Immunoglobulins

classes of, 363

class switching, 371

evolution of, 372

functional structure, 362, 363

genetic basis of diversity in, 368-371

IgG class, 363-364

See also Antibodies Immunological memory, 359, 360 Immunological tolerance, 359,

360-362, 367-368 Imperfect flowers, 522 Imperfect fungi, 530, 539 Impermeable membranes, 85-86 Implantation, 742, 758

contraceptives and, 747

hormonal response to, 745

time of, 769 Imprinting, 929-930 Inbreeding, 188

eusociality and, 955 Incisors, 894, 895 Inclusive fitness, 954 Incomplete cleavage, 757 Incomplete dominance, 186-187

Incomplete metamorphosis, 572,

715 Incus, 802, 803 Indeterminate cleavage, 545 Indeterminate growth, 612, 670 Indian pipe, 643-644 Indirect transduction, 285 Indoleacetic acid (IAA). See

Auxin Indri indri, 1041 Induced mutations, 236, 237 Inducers, 250, 301-302 Inducible enzymes, 250 Inducible promoters, 323 Induction, 300

in embryo development, 301-302 Inductive interactions, 759 Industrial nitrogen fixation,

641-642 Infants, genetic screening,

340-341 Infarctions (cardiac), 874 Infections

diagnosing with PCR, 329

fevers and, 709

lymph nodes and, 877

opportunistic, 373 Infectious diseases

bacterial, 466-467

group living and, 953

Koch's postulate, 467 Inferior vena cava, 871 Inflammation, 357-358

causes of, 876-877 Inflorescence meristems, 670, 671 Inflorescences, 522, 670 Influenza virus

reproductive cycle, 243

size range, 240 Information, central dogma of molecular biology, 220-221

Infrared radiation, 139 detection in snakes, 812

Inhalation, 858, 859, 863-864. See also Tidal breathing

Inheritance

blending theory, 177, 180 cytoplasmic, 196 Mendel's experiments,

178-180, 181-182 Mendel's laws, 180-183 particulate theory, 180 sex-linked, 195-196 See also Genetics

Inherited behavior adaptive value of, 930 experimental studies, 927,

928-930, 932-934 in humans, 944 imprinting, 929 releasers, 927-928, 929 spatial learning, 928-929 stereotypic and species-specific, 926

Inhibition

end-product inhibition, 110 enzyme inhibitors, 106-107,

108-109 G protein-mediated, 284

Inhibitory postsynaptic potential (IPSP), 787, 788

Inhibitory synapses, 786-787

Initial bodies, 472

Initiation complex, 228-229

Initiation factors, 229

Initiation sites, 223

Innate defenses, 355

complement proteins, 356-357 inflammation, 357-358 interferons, 357 overview of, 353-354 phagocytosis, 357 summary of, 356 surface barriers, 356

Inner cell mass, 758, 764

Inner ear, equilibrium organs in, 801-803

Inoculations, 353, 359

Inorganic fertilizers, 639

Inositol triphosphate (IP₃), 288

Insecta, 571, 578

Insecticides, transgenic plants and, 325-326, 684

Insectivores, 595

Insect-pollinated flowers, 524

Insects

cleavage in, 757 development in, 295 diversity in, 571-572, 573 eusocial, 954-955 exoskeleton, 842-843 fertilization in, 737 gas exchange systems, 853 heat production in, 704 juvenile hormone and,

716-717 major lineages, 572, 574 Malpighian tubules, 914-915 molting, 564, 715-716 structure of, 572

Insert mutations, 369

Insomnia, 825

Inspiratory reserve volume, 856,

857 Instars, 572, 715 Insulin, 723-724

biotechnology and, 323, 325

receptor for, 283-284

regulation of blood glucose, 904-905

targets and actions of, 718 Insulin-dependent diabetes, 372,

729 Integral membrane proteins, 80,

82,87-88 Integrase, 374 Integrated pest management

(IPM), 998-999 Integument, 517, 519 Intercalated discs, 834 Intercostal muscles, 858-859 Interference competition, 978 Interferons, 357 Intergroup mutualism, 983 Interkinesis, 270, 171 Interleukins, 159, 368

in B cell class switching, 372

fevers and, 709 Intermediate filaments, 72, 74 Internal environment, 694 Internal fertilization, 737 Internal fibrils, 462 Internal gills, 852, 853-854, 855 Internal intercostal muscles, 859 Internal membranes, in cell evolution, 479 Internal skeletons. See Endoskeletons Internal-transcriber-spacer (ITS),

432 International Olympic Committee, 727 Interneurons, 818 Internodes, 604, 611, 672 Interphase

meiosis, 170

mitosis, 157-158, 160, 161, 262 Intersex individuals, 726 Interspecific competition, 976,

977 Intertropical convergence zone,

992,1014 Intestinal bacteria, 902 Intestines, 895 Intracytoplasmic sperm injec-

' tions (ICSI), 749 Intraspecific competition, 976,

977-978 Intrauterine device (IUD), 746, 747 Intrinsic factor, 893 Inrrons, 265, 266, 267, 268, 269 Inuit peoples, 892 Inversions, 235, 236 Invertebrates

defense systems, 372

excretory systems, 914-915

visual systems, 807 In vitro fertilization, 748-749 Involuntary nervous system, 815 Involution, 760 Iodine

in animal nutrition, 890

thvroid gland and, 893

thyroxine and, 722

lodyticium 5 >« Ion channels, 28 ;

effects on membrant potential

facilitated diffusion, 87-88

ionotropic rocepto

790 in long-term potentiation, 827 resting potential and, 778 sense of smell and, 290-291 in sensory receptors, 795 in sensory transduction,

signal transduction and, 290 studies of, 783 See also Calcium channels; Potassium channels; Sodium channels; Voltage-gated channels

Ion exchange, 639

Ionic bonds, 21, 24

Ionic conformers, 912

Ionic regulators, 912

Ionizing radiation, 236

Ionotropic receptors, 788, 789-790

Ion pumps, 778

Ions, 18, 24

absorption in large intestine, 901-902

IPM. See Integrated pest management

Iris (eye), 808

Iron, 277, 634

in animal nutrition, 890, 891 in plant nutrition, 636

Iron deficiency (plant), 636

Ischemias (cardiac), 874

Ischium, 843

Island biogeographic model, 1012-1014

Islands, extinction rates on, 1031

Islets of Langerhans, 724

Isocitrate, 223, 124

Isocitrate dehydrogenase, 133

Isogamous life cycle, 493

Isoleucine, 37, 224, 889, 890

Isomers, 32

Isomorphic life cycle, 490, 493

Isoprene, 51

Isoptera, 572

Isotonic solutions, 87

Isotopes, 19

Isozymes, 112

Isthmus of Panama, 1027

IUD. See Intrauterine device

Ivanovsky, Dmitri, 240

Ixodes ricinis, 570

Jasmonates, 647, 660, 683, 684 Jaw joint, 846

Jawless fishes, 583, 584, 585 Jaws, evolution in fishes, 584,

585 Jefferson, Estlin, 329 Jefferson, Thomas, 329 Jejunum, 897, 899 Jellyfish, 218, 317, 548, 549, 550,

846 rter, Edward, 353

Jews

penile circumcision and hemophilia, 176

Tay-Sachs disease and, 341 Joints

in endoskeletons, 845-846

in exoskeletons, 842 Jones, Marion, 773 Joshua tree, 986 Joules, 26, 887 Jumping, 831 Junipers, 520-521 Jurassic period, 380-381, 389, 521 Juvenile hormone, 716-717

Kalanchoe, 672, 674, 676 Kale, 399

Kangaroo rats, 930 Kangaroos, 594, 2007 Kaposi's sarcoma, 343, 373 Karyogamy 533 Karyotypes, 167 Katydid, 573 Kelps, 489

Kentucky blue grass, 150 Keratins, 39, 85, 296 a-Ketoglutarate, 123,124,131 a-Ketoglutarate dehydrogenase, 124-125 Ketones, 31 Keystone species, 986 Kidney beans, 670 Kidney dialysis, 324 Kidneys

antidiuretic hormone and, 718

blood vessels, 919-920

buffering functions of, 924

concentration of urine, 920-921

control and regulation of, 922-924

erythropoietin production in, 880

functions of, 918-919

glomerular filtration rate, 920

nephron structure and function, 915-916, 917, 919

Wilm's tumor, 345 Kidston, Robert, 508 Kilocalories, 26 Kimura, Motoo, 439 Kinases, 119 Kinesins, 75, 718 Kinetic energy, 96 Kinetochore microtubules, 262,

163 Kinetochores, 262, 163, 164 Kinetoplastids, 477, 483, 484 Kinetoplasts, 483 King, Thomas, 297 Kingdoms, 434 Kinorhynchs (Kinorhyncha), 565,

578 Kin selection, 954 Kirtland's warbler, 1039 Kiwis, 447, 448 Klinefelter syndrome, 194 Kliugin, Sergey, 832 Knee-jerk reflex, 817, 845 Knee joint, 845, 846

Knife fish, 847

Knockout experiments, 320, 321

Knots, 617

Knudson, Alfred, 345

Koalas, 893

Koch, Robert, 466-467

Koch's postulate, 467

Kohlrabi, 399

Kolreuter, Josef Gottlieb, 177

Kornberg, Arthur, 206

Krakatau, 383-384,1013

Krause's end bulb, 800

Krebs, Edwin, 287

Krebs cycle. See Citric acid cycle

Kriippel gene, 307

Kurosawa, Eiichi, 651
 Kuru, 334
 Kwashiorkor, 892, 893
 Labia majora, 741, 742
 Labia minora, 741, 742
 Labidomera clivicollis, 685
 Labor contractions, 770-771
 Lacewings, 572
 Lacks, Henrietta, 155
 lac operon, 251-252, 254, 317
 Lactase, 901
 Lactate, 104, 129
 Lactate dehydrogenase, 104
 Lactic acid fermentation, 129
 Lactose, 250, 251, 901
 Lactose metabolism
 lac operon, 251-252, 254
 in prokaryotes, 250-252 Laetiporus sulphureus, 538 Lagging strand, 211-212 Lakes, 1027
 cycling of materials, 999-1000
 eutrophication, 1004-1005
 food web, 996
 PCBs and, 908 Lamarck, Jean Baptiste de, 2 Lambda phage, 254, 255, 316 Lamellae, of internal gills,
 853-854, 855 Laminaria, 490 Lamins, 63
 Lampreys, 583, 584, 737 Lamp shells, 578 Lancelets, 582, 583 Land, vertebrate colonization of,
 587-591 Land snails, 422, 562, 562 Landsteiner, Karl, 187 Lang, William H., 508 Language
 brain areas involved in, 828-829
 human, 598-599 Langurs, 445, 446 Laqueus, 557
 Large intestine, 356, 897, 901-902 Larvae, 717
 evolutionary relationships and, 430, 432 Larynx, 857, 858, 898 Lascaux Cave drawings, 599 Lateral buds, 605 Lateral gene
 transfer, 468 Lateral hypothalamus, 906
 Lateralization, of language functions, 828 Lateral line sensory system, 801,
 812 Lateral meristems, 612, 613
 in secondary growth, 615-616, 617 Laticifers, 684, 685 Latimeria chalumnae, 587 Laurasia, 380, 1008 Law of independent
 assortment,
 182-183 Law of mass action, 30 Law of segregation, 180-182 LDLs. See Low-density lipoproteins Leaching
 seed germination and, 649, 650
 in soil, 638, 639 Lead, 906
 Leading strand, 211 Leafhoppers, 572 Leaflets, 606 Leaf primordia, 614-615 leafy gene, 304, 305 Learned behavior
 culture and, 925

humans and, 944

in macaques, 925 Learning

bird songs and, 929-930

brain areas involved in, 826-827

glutamate receptors and, 789-790

spatial, 928-929 Leaves, 604, 612

abscisic acid and, 660

abscission, 655, 658

adaptations to dry environments, 685-686

anatomy of, 249, 617-618

calcium-induced calcium release in, 289

in C 3 and C 4 plants, 249

chloroplasts in, 69

chlorosis, 636

definition of, 510

development of, 614-615

evolution of, 520

in flowering, 648

foliar fertilizer sprays, 639

guttation, 624, 625

of halophytes, 688

homologous structures derived from, 428

length of night and, 661

parasitic fungi and, 531, 532

simple and complex, 510-511

structure of, 605

types of, 605-606

vegetative reproduction and, 676 Lederberg, Joshua, 245-246 Leeches, 95, 558-559, 895-896 Left-right axis, determination of,

756 Leghemoglobin, 641 Legumes, nitrogen fixation in, 640, 641, 642

Leishmaniasis, 483 Lemond, Greg, 196 Lens, 808

development, 301-302

gap junctions between cells, 292

of ommatidia, 807 Lens placode, 301 Lenticels, 617 Leontopithecus rosalia, 596 Lepas pectinate, 570 Lepidodendron, 512 Lepidoptera, 574 Lepidosiren, 432 Lepidosirendids, 432 Lepisma saccharina, 573 Leptin, 906 Leptoid cells, 506 Lesser bush baby, 595 Leucine, 37, 224, 889, 890 Leucine zipper motif, 273 Leucoplasts, 70, 608 Leucospermum conocarpodendron, 1010 Leucothea, 552 Leukemias, 259, 343 Levers, 845-846 Lewis, Edward, 307 Leydig cells, 740, 741 Lice, 574

Lichens, 532, 540-542, 983 Liebig, Justus von, 114 Life

atmospheric oxygen and, 380, 382, 454-455, 466

elsewhere than Earth, 455-456

emergence of, 3

essential characteristics of, 451

longer evolves from nonlife, 455

mechanistic view of, 17

origins of, 450-454

temperature and, 699-700

water and, 17

Life cycles

alternation of generations, 489-490

haplontic and diplontic, 166, 49⁴494

heteromorphic and isomorphic, 490, 493

isogamous and anisogamous, 493

Life histories

defined, 967

reproductive value, 969-970

stages in, 968

trade-offs, 968-969

traits of, 968

using in population management, 970-972

Life tables, 962-963

Ligaments, 845

Golgi tendon organs and, 800, 801

Ligands, 41, 282

Light

absorption by pigments, 139-140

action and absorption spec-trums, 140-141

in photosynthesis, 136, 137

physical properties, 138-139

in plant development, 646, 647, 648, 661-663

Light chains, of immunoglobulins, 362, 363, 369

Light intensity, 139

Light microscopes, 56, 57

Light reactions

cyclic electron flow, 144-145

light absorption, 142-143

noncyclic electron flow, 143-144

overview of, 137, 138

photophosphorylation, 145-146

Lignier, E. A. O., 510

Lignin, 503, 607, 679

Limbic system, 818-819, 828

Limbs

development, 305

evolution in mammals, 3

mechanisms of movement, 817-818

phantom, 795

whale evolution, 385

Limes, 891

Liming, 639

Limiting resources, 975-976

Limulus polyphenols, 569

Linanthus, 432

Lind, James, 891

LINEs, 264-265

LIN-3 growth factor, 302

Linickia, 733

Linkage groups, 191

Linked genes, 190-191

Linnaeus, Carolus, 414, 433, 1008

Linnean classification, 433-434, 435

Linoleic acid, 891

Lionfish, 981

Lions, cooperative hunting, 953

Lipases, 896, 899, 902

Lipids

in archaea membranes, 472 carotenoids, 51-52 catabolic interconversion, 131 fats and oils, 49-51 functions of, 49 in membranes, 51, 79-81, 472 physical properties, 49 second messengers derived

from, 287-288 steroids, 51, 52 vitamins, 52 waxes, 52-53 See also Phospholipids

Lipid-soluble hormones, 728

Lipofection, 317

Lipoproteins, 91, 333, 904, 905

Liposomes, 317

Littoral zone, 1026

Liver, 897

bile and, 899, 900 edema and, 877 lipoproteins and, 904, 905 in regulation of fuel metabolism, 904

Liver cancer, 343

Liverworts, 501

characteristics of, 502, 503, 504-505

mycorrhizae and, 540 Lizards, 589

fertilization in, 738

parthenogenetic reproduction in, 734

thermoregulation in, 701-702 Llama guanaco, 861 Llamas, 600, 861-862 Load arms, 845-846 Loam, 638

Lobe-finned fishes, 587 Lobelia, 1038 Lobsters, 543, 801 Locomotion. See Cell movements; Motility Lofenelac, 346 Logistic growth, 964-965 Lolium perenne, 976, 977 Long-chain hydrocarbons, 473 Long-day plants, 671-672 Long interspersed elements

(LINEs), 264-265 Longistigma caryae, 629 Longitudinal muscle layer, 897 Long-short-day plants, 672 Long-term depression (LTD), 827 Long-term memory, 819, 828 Long-term potentiation (LTP),

791, 792, 826-827 Loop of Henle, 919, 920-921, 922 Loose connective tissue, 696 Lophodermium, 988 Lophophorates, 555-557 Lophophores, 555, 556, 557 Lophotrochozoans

flatworms, 553-554

general characteristics, 574

major lineages, 555

phylogeny, 553

rotifers, 554-555

subgroups and number of living species, 578 Lordosis, 931 Lorenz, Konrad, 927, 929 Low-density lipoproteins

(LDLs), 91, 333, 904, 905 Loxodonta africana, 166 Luciferase, 317 Lucy (australopithecine), 597 Lumbricus, 559 Lumen, 897 Lung cancer, 347 Lungfishes, 432, 433, 587, 869 Lungs, 852

of birds, 854-856

evolution in lungfishes, 869

tidal breathing, 856-857

See also Respiratory system Lunularia, 505 Lupus, 359, 372 Luscinia svecica, 952 Luteinizing hormone (LH), 718, 719

male sexual function and, 741

in ovarian and uterine cycles, 743, 744, 745

in puberty, 726, 727 Lycoperdon perlatum, 538 Lycophyta, 502, 510, 511-512 Lycopodium obscurum, 512

Lycopods, 508, 511-512 Lyell, Charles, 379 Lygodium microphyllum, 513-514 Lymph, 354, 877 Lymphatic system, 698, 877

fat absorption and, 901 Lymph nodes, 354, 877

in HIV infection, 373 Lymphocytes

daughter cells, 360

in lymph nodes, 877

overview of, 354

types of, 355

See also B cells; T cells Lymphoid progenitor cells, 355 Lymphoid tissues, 354 Lymphomas, 343, 373 Lynx, 979

Lyperobius hutttoni, 1010 Lysine, 37, 224, 889, 890 Lysogenic cycle, 241, 242, 254,

255 Lysosomes, 63, 66-67 Lysozyme, 105, 106, 356

evolution in, 445-446

tertiary structure, 40 Lytic cycle, 241, 242, 254, 255

Macaca

M. mulatta, 167

M. sylvanus, 596 Macaques, 596, 925 MacArthur, Robert, 1012 MacLeod, Colin, 201 Macrocystis, 477 Macroevolution, 379-380 Macromolecules

analyzing evolution in, 440-441

carbohydrates, 43-46

condensation reactions, 35

defined, 34

functional groups, 35

functions of, 34-35

interactions of, 53

lipids, 49-53

nucleic acids, 47-49

proteins, 36-42

X ray crystallography, 440 Macroneustes gigantus, 913 Macronuclei, 485-486, 487 Macronutrients

in animal nutrition, 890, 891

in plant nutrition, 635, 636 Macrophages
 as antigen-presenting cells, 354, 366
 cytokines and, 358
 functions of, 355, 357, 358, 368
 HIV and, 373, 374 *Macropus rufus*, 594 *Macrosiphum rosae*, 733 Madagascar
 endemic species, 1040, 2042
 prosimians of, 595 *Madia sativa*, 422 Magma, 450 Magnesium, 634
 in animal nutrition, 890
 NMDA channels and, 790
 in plant nutrition, 636
 in soils, 638
 Magnesium deficient) (plant),
 636 Magnetism, 2
 bird navigation and, 943 Magnetite Magnolia S23 525 Magpies, 395 406 Maidenhair fern, 51 1 Maidenhair tree 518 >29
 Maimonides, Moses, 176 Maize 5 C om Major histocompatibility complex (MHC) proteins
 antigen-presenting roles of, 366 ^~.36S
 classes of, 366
 in immunological self-tolerance, 367-368
 overview of, 355
 in transplant rejection, 368 Major histocompatibility gene
 complex, 367, 372 *Malacosoma californicum*, 978-979 Malaria, 413, 485 Malate, 223, 124 Malathion, 791 Male genitalia, 740
 Males
 hormonal control of sexual behavior, 931-932
 mating behavior and, 950, 951, 952
 parenting behavior, 956
 sex organs, 739-741 Malic acid, 151 Malignant tumors, 343 Mallard ducks, 926-927 Malleus, 802, 803 Malnutrition, 889
 Malpighi, Marcello, 629 Malpighian tubules, 914-915 Maltase, 901 Malting, 650 Maltose, 44 Mammals (Mammalia), 578
 circadian clock, 937-938, 939
 circulatory system, 870-871
 cleavage in, 757, 758
 diving reflex, 884
 domestication of, 1028
 excretory system, 918-922
 forelimb evolution, 3
 gastrulation in, 764-765
 lungs and respiratory gas exchange, 856-859
 origin and diversity of, 593-594
 placenta, 768
 sex determination, 194
 teeth, 894, 895

uterus, 739 Mandibles, 843, 895 Manganese

in animal nutrition, 890

in plant nutrition, 636 Manganese deficiency (plant),

636 Mangold, Hilde, 762 Mangroves, 687, 688

risks of, 1013-1014 Mannose, 44 Mantids, 572

Mantle, of mollusks, 560

Maple trees, 620 Map units, 192 Marine invertebrates, 505

Marine communities/ecosystems chemosynthetically powered,

998 dispersal of organisms,

1026-1027 mass extinctions, 382, 383,

391-392 ocean zones, 1026, 2027 predators in, 986-987 vicariant events, 1027 Marine iguanas, 703, 704 Marine invertebrates, as osmo-

conformers, 911 Marine mammals, diving reflex,

884 Marine reptiles, in evolution, 393 Marine worms, ecdyspzoan,

565-566 Maritime climates, 994 Marsileales, 514 Marsilea mutica, 513 Marsupials (Marsupialia),

593-595, 739 Marsupium, 739 'Maryland Mammoth' tobacco,

671, 672 Masai people, 599 Mass, 17-18

distinguished from weight, 19n Mass extinctions, 382, 382, 383, 384, 387, 388, 389, 390, 391-392 Mass number, 19 Mastax, 555

Mast cells, 355, 357, 358, 713, 876 Maternal effect genes, 305-306 MAT gene, 275 Mating behaviors

courtship displays, 926-927 female behaviors, 952 hybridization experiments,

927 male attraction of mates, 951 molecular genetic studies in

Drosophila, 934 sexual selection and, 950-951 social and genetic partners,

952 sperm and egg costs in, 950 Mating systems, 736-737, 738 Mating types, in fungi, 533 Matthaei, J. H., 224-225

Maturation promoting factor, 158 Maxicircles, 483 Maxilla, 843 Maximum likelihood method,

430 Mayflies, 572 Mayr, E- nst, 414 M band, 836, 837 McCarty, Maclyn, 201 Meadowlarks, 423 Mechanical weathering, 638

Mechanoreceptors, 795

in auditory systems, 803-804 in equilibrium organs, 801-803 properties and actions of, 799

stretch, 800, 801

tactile, 799-800 Mechanosensors, 795 Medicine

biotechnology and, 323-325

comparative genomics and, 262

Koch's postulate, 467

PCR applications in, 329

potential use for stem cells in, 294, 299, 300

use of leeches in, 559 Medulla (brain), 816

in breathing, 863, 864

regulation of blood pressure and, 882, 883, 884

reticular system and, 818 Medulla (kidney), 919 Medusa, 549, 550 Megacalanus princeps, 570 Megagametophytes, 511, 666, 667 Megakaryocytes, 880 Megapascals, 621 Megasporangia, 666, 667

angiosperm, 522

conifers, 519

seed plants, 516-517 Megaspores, 511

conifers, 518

ferns, 514

seed plants, 516 Megasporocytes, 666, 667 Meiosis

chromatin, 160, 268, 171

emergence of, 5

first meiotic division, 167, . 168-169, 170-171

functions of, 167

genetic diversity, 172

independent assortment of alleles, 283

meiotic errors, 172-173

mitosis compared to, 170-171, 171-172

mutations during, 236

overview of, 157

second meiotic division, 167, 168-169, 171

segregation of alleles, 282

sexual reproduction, 165, 266 Meiosis 1, 167, 168-169, 170-171,

172 Meiosis II, 167, 168-169, 171 Meissner's corpuscles, 799, 800 Melanin, 189, 332 Melanin chagresi, 996 Melanocyte-stimulating hormone, 728, 719, 720 Melanocyte-stimulating hormone release-inhibiting hormone, 722 Melatonin, 729, 727, 728 Membrane lipids, of archaea, 472 Membrane phospholipids, 51, 79-81

as second messengers, 287-288 Membrane potential, 622

defined, 776

depolarization and hyperpolarization, 779, 780

ion pumps and channels,

777-779 measuring, 777 Nernst equation and, 779 photosensitivity and, 806 physics of, 777 in sensory transduction,

795-796 smooth muscle contraction

and, 834 See also Action potential; Resting potential Membrane proteins

asymmetric distribution,

81-82 carrier proteins, 88 in cell adhesion, 83-84 facilitated diffusion, 87-88 integral, 80, 82 ion channels, 87-88 peripheral, 80, 82 transmembrane, 82 Membranes of archaea, 472 carbohydrates in, 82 cell adhesion, 82-84 cell junctions, 84-85 and chemical reactions, 92, 93 composition and structure,

79-82 diffusion across, 85-86 dynamic nature of, 92-94 endocytosis and, 90-91 energy transformation and, 92,

93 exocytosis and, 90, 91-92 fluid mosaic model, 79-82 freeze-fracture technique with,

82 information processing and,

92,93 osmosis and, 86-87, 621 permeable and impermeable,

85-86 phospholipids in, 51, 79-81 proteins in, 81-82 See also Plasma membrane Membrane transport active, 88-90

passive processes, 85-88, 89 Membranous bone, 844 Membranous compartments, 62.

See also Organelles Memory

brain areas involved in,

827-828 glutamate receptors and,

789-790 hippocampus and, 819 types of, 828 Memory cells, 360, 363 Menadione, 892 Mendel, Gregor

experiments of, 176, 178-180,

181-182 laws of, 180-183 neglect of work by, 177-178 Mendelian genetics

law of independent assortment, 182-183 law of segregation, 180-182

Mendel's experiments, 178-180, 181-182

See also Genetics; Inheritance Mendelian populations

allele frequencies, 399-400

defined, 399

Hardy-Weinberg equilibrium, 399-400 Menertea, 578 Menopause, 742 Menstruation, 743-745 Mental retardation

fragile-X syndrome, 335-336

phenylketonuria, 331, 332, 340, 346 Mercaptan, 847 Mercury pollution, 991 Meristems

apical, 612-613, 614, 670-671

cell divisions in, 612

floral, 304, 670-671

lateral, 612, 613

in root growth, 613

in secondary growth, 613, 615-616, 617

in shoot growth, 614-615

tissue culturing, 677 Merops bullockoides, 954 Merostomata, 569, 578 Merozoites, 485 Mertensia virginica, 433 Meselson-Stahl experiment, 206,

207-208 Meselson, Matthew, 207 Mesenchyme, 544, 545 Mesocoel, 555 Mesoderm, 545

extraembryonic membranes and, 768

fate of, 759

formation of, 759, 760, 762

in neurulation, 765 Mesoglea, 549 Mesonychid, 385 Mesophyll, 618 Mesosomes, 59, 462, 555 Mesozoic era, 1008

evolutionary trends in, 389-390

major events in, 380-381

plant evolution in, 518 Mesquite trees, 687 Messenger RNA (mRNA)

alternate splicing, 276

analyzing with DNA chip technology, 321

central dogma and, 221

codons, 224

G caps, 277

primary transcript processing, 268-270

regulation of life span, 276

transcription of, 222

translational control of, 276-277

translation of, 225-231 Metabolic compensation, 700 Metabolic factors, 249 Metabolic inhibitors, 346 Metabolic pathways

allosteric regulation, 110, 222, 132-134

anabolic and catabolic, 108, 131-132

commitment step, 110

consequences of undernutrition, 132

end-product inhibition, 110

evolutionary history, 134

interconversions, 131-132

in plants, 151-152

See also Glucose metabolism Metabolic rates

basal, 705, 706, 887-888

energy yields from food and, 887-888

temperature and, 700

thermoregulation in endotherms, 706 Metabolic regulation

allosteric, 132-134

transcription-level, 250 Metabolism, 4

in coacervates, 453

defined, 95

governing principles, 114

in prokaryotes, 464-466

in proteobacteria, 469

thyroxine and, 722

types of metabolic reactions, 96

waste products of, 923

See also Fuel metabolism; Glucose metabolism; Metabolic pathways Metabotropic receptors, 788-789 Metacarpal bones, 843 Metacoel, 555 Metal tolerance, plants and, 418,

689 Metamorphosis, 5-6, 572

complete, 726, 717

incomplete, 715 Metanephridia, 914, 925 Metaphase

karyotyping, 167

meiosis, 268, 269, 171

mitosis, 263, 164 Metasome, 555 Metastasis, 343 Metatarsal bones, 843 Meteorites, 384, 390 Methane, 22

intestinal bacteria and, 902

from methanogens, 473

ruminants and, 902-903 Methanogens, 473-474 Methanopyrus, 474 Methanospirillum hungatii, 460 Methionine, 229, 889, 890

genetic code for, 224

plant sulfur metabolism and, 643

side chain, 37 Methylases, 313 Methylation, 274-275, 312 5-Methylcytosine, 338 Methyl salicylate, 681 Mevinolin, 347 Mice

coat color in, 188

gypsy moths and, 989

knockout experiments, 320, 321

ob/ob mutation, 906

Micelles, 900 Micrasterias, 492 Microcentrum rhombifolium, 573

Microevolution

associative mating, 404

defined, 379

gene flow, 402

genetic variation and, 408-409

germ-line mutations, 402

methods of studying, 406-408

phenotypic plasticity and, 410

random genetic drift, 402-404

results of natural selection, 404-406 Microfibrils, cellulose, 656-657 Microfilaments, 831

in cytokinesis, 164

structure and function, 72-73 Microgametophytes, 511, 666,

667 Micronuclei, 485-486, 487 Micronutrients

in animal nutrition, 890, 891

in plant nutrition, 635, 636 Microorganisms

sizes of, 240

See also Bacteria; Prokaryotes Microparasite-host interactions,

978-979 Microparasites, 978 Micropyle, 519 Microsatellites, 263 Microscopes, 56-57 Microspheres, 453 Microsporangia

angiosperm, 521

seed plants, 517 Microspores, 511

conifers, 518

ferns, 514 Microtubule organizing center,

74 Microtubules, 831

centrioles, 75-76

in cilia and flagella, 74-77, 832-833

in cytoskeletal movement, 833

mitotic spindle, 161, 262, 163

structure and function, 72, 74 Microvilli, 73

intestinal, 896

microfilaments in, 833

in taste buds, 798

See also Stereocilia Midbrain, 816 Middle ear, 802, 803

deafness and, 804 Middle lamella, 607 Midgut, 895 Migration, 966-967

gene flow and, 402

navigation in, 941-943

in population dynamics, 962 Migratory restlessness, 941 Milk, 593 Milkweeds, 685 Miller, Stanley, 452 Millipedes, 571 Mimicry, 980, 982 Mineralcorticoids, 729, 725, 726 Mineral nutrients

in animal nutrition, 890, 891

ion exchange, 639

overview of, 634

in plant nutrition, 635-637

in soil, 638

uptake and transport in

plants, 620-626

Mine tailings, plants and, 418,

689 Minicircles, 483 Minimal genomes, 256-257 "Mini-pill" (contraceptive), 746 Minisatellites, 263 Mirounga angustirostris, 403 Miscarriages, 747 Mismatch DNA repair, 213 Missense mutations, 234-235 Mistletoes, 643 Mites, 734, 737 Mitochondria, 67

in animal cells, 60

cytoplasmic inheritance, 196

egg contributions to zygotes, 754

endosymbiosis and, 70, 479

function of, 63

genetic code and, 224

mutation levels, 196

organisms lacking, 479

photorespiration in, 149

pyruvate oxidation in, 122, 223

respiratory chain, 125-129

structure and function, 68 Mitochondrial DNA (mtDNA)

human evolution and, 438, 448

in phylogenetic studies, 431 Mitochondrial matrix, 68 Mitosis

in asexual reproduction, 165

cell cycle and, 158

chromatin in, 160, 262, 163

meiosis compared to, 170-171, 171-172

orientation and centrosomes, 160-161

overview of, 157, 160

phases in, 162-163, 163-164

polyploidy and, 173

spindle formation, 161, 262, 163 Mitotic centers, 161 Mitotic spindles, 757-758 Mniium, 505 Moas, 447, 448 Moderately repetitive sequences,

263-264 Modules, 960

in plant development, 611 Molars, 894, 895 Molds, 536 Molecular biology, central

dogma, 220-221 Molecular clocks, 439, 441-442,
 937-939, 940 Molecular evolution
 analyzing macromolecules, 440-441
 defined, 438
 gene duplication and gene families, 442-445
 genome size and noncoding DNA, 446-447
 homologous genes, 444-445 molecular clocks 439, 441-442 neutral theory of, 439-440 nucleotide substitutions and,
 441 protein evolution, 445-447 ribosomes and, 447, 448
 Molecular formulas
 Molecular mass
 Molecular phylogenetics, 438 evolution in flightless birds,
 447-448 human evolution, 447-448 prokaryote evolution and,
 using rRNA in, 447 Molecular traits, 430-431 Molecular weight, 28 Molecules, 8
 defined, 20
 functional groups, 31-32
 ground and excited states, 139-140
 isomers, 32
 properties of, 30-32
 weights and sizes, 29 Moles (animal), 595 Moles (chemistry), 28 Mollusks (Mollusca), 559-562
 body plans, 560
 cilia, 831, 832
 circadian clock in, 938
 cleavage in, 758
 general characteristics, 574
 habitat selection, 948-949
 nervous system, 774
 open circulatory system, 867
 subgroups and number of living species, 578
 toxins from, 847 Molting
 in animals, 564, 843
 in insects, 564, 715-716 Molybdenum
 in animal nutrition, 890
 in plant nutrition, 636 Monadenia fidelis, 561 Monarch butterflies, 6, 967 Monkeys, 595-596 Monoamines, 790 Monoclonal
 antibodies, 364, 365 Monocots (Monocotyledons), 526, 527, 604
 root anatomy, 624
 shoot development patterns, 648
 stem thickening in, 617
 vascular bundles, 625 Monocytes, 355, 357 Monod, Jacques, 252 Monoecious flowers, 522 Monoecious organisms, 192, 736
 Monoglycerides, 901 Monohybrid crosses, 278,

179-180, 184 Monomers, 34, 35 Monophyletic groups, 435, 436 Monoplacophorans
'Monoplacophora), 560, 578 Monosaccharides, 43–44 osomy 272, 173

Monos) rtaptic reflex, 817 Monoterpenes, 683 Monotremata, 593 Monounsaturated fatty acids, 50 Monozygotic twins, 759
Monteverde Cloud Forest

Reserve, 587 Moraines, succession on, 987-988 Morchella esculenta, 536 Morels, 536 Morgan, Thomas Hunt, 190,192,
195 Morphogenesis

in development, 295

pattern formation and, 302 Morphogens, 305 Mortality rates. See Death rates Morus bassanus, 951 Mosaic development,
759 Mosquitoes

chromosome pairs, 267

malaria and, 485

speciarion in, 413 Mosses, 500, 502

archegonia and antheridia of, 505

characteristics of, 502, 503, 506-507 Moths, 574

adult stage, 968

metamorphosis in, 726, 717

mimicry, 982

sex determination, 194

species-specific coevolution and, 985-986 Motifs, 273 Motility

ciliates, 486, 487

in ciliates, 486, 487

as growth in plants, 635

hydrostatic skeletons and, 841-842

prokaryotes, 462-463

in prokaryotes, 462-463

protists, 480

in protists, 480

See also Cell movements Motor end plates, 785, 786, 838 Motor neurons

motor units and, 838

neuromuscular junction, 785-786 Motor proteins

in cilia and flagella, 75

microtubules and, 74 Motor units, 838, 840 Mountains, species richness in,
1014 Mouth, 895

in deuterostomes and proto-stomes, 545

digestion in, 898 Movement proteins, 293 M phase, 158. See also

Cytokinesis; Mitosis mRNA. See Messenger RNA Msfl restriction enzyme, 341,
342 mtDNA. See Mitochondrial DNA Mucopolidosis II, 233 Mucosa, 897, 899, 903

Mucus, 897

innate defenses and, 356

in lungs, 858

olfaction and, 798 Mucus escalator, 858 Mud puppy, 809 Mule deer, 682 Mules, 429

Mullerian mimicry, 980, 982 Multicellularity, nematode genes

essential to, 262 Multicellular organisms, emergence and development of, 5-6 Multifactorial diseases, 335 Multiple fruits, 525 Multiple sclerosis, 372 Murgantia histrionica, 573 Musca domestica, 167 Muscarine, 788 Muscarinic receptors, 788, 789 Muscle cells

microfilaments in, 73

neuromuscular junction, 785-786 Muscle contraction

actin and myosin filaments in, 833-834, 836, 837-839

calmodulin and, 289, 839

in cardiac muscle, 834-835

fast-twitch and slow-twitch fibers, 840-841

in skeletal muscle, 835-839

in smooth muscle, 834, 835

summing of muscle twitches, 839-840

tetanus, 840

tonus, 840 Muscle fibers, 300

in arteries and arterioles, 875, 876

chromatophores and, 846-847

contraction of, 837-839

fast-twitch and slow-twitch, 840-841

motor units and, 838

structure of, 836, 837

in veins, 878 Muscles, 695, 697, 698

anabolic steroids and, 727

ciliary, 808

flexor and extensor, 817-818, 845

intercostal, 858-859

joints and, 845

oxygen-myoglobin binding properties, 861

skeletons and, 841-846

stretch receptors and, 800, 801

types of, 834

See also Cardiac muscle;

Skeletal muscles; Smooth muscles Muscle spindles, 800, 801 Muscle twitches, 839-840 Mushrooms, 538 Mussels, 987

Mutagenic chemicals, 236, 237 Mutation rates, 369, 402 Mutations

in alleles, 186

cancer and, 343-344

chromosomal, 234, 235-236

defined, 233

detecting with DNA chip technology, 321-322

evolution and, 236-237

frequency of, 236

germ-line, 234

identifying genes responsible for, 336-339

induced, 236, 237

5-methylcytosine and, 338

in microevolution, 402

neutral, 408

in organelle genomes, 196

origin of life and, 452, 453

point, 234-235

in prokaryotes, 468

in protein targeting, 233

restrictive and permissive conditions, 234

somatic, 234

spontaneous, 236, 237

synthetic, 319-320 Mutualisms, 975

animal-animal, 984

human disruption of, 1038

intergroup, 983

in nitrogen fixation, 640, 641

plant-animal, 984-985 Mycelium, 531 Mycobacterium tuberculosis, 256, 472 Mycoplasma

M. gallisepticum, 472

M. genitalium, 256-257, 446 Mycoplasmas, 240, 472 Mycorrhizae, 532, 539-540, 614, 641, 983 Myelin, 776, 784

multiple sclerosis and, 372 Myeloid progenitor cells, 355 Myelomas, 364, 365 Myoblasts, 300 MyoDl gene, 300 MyoDl protein, 300 Myofibrils

in muscle contraction, 837-839

structure of, 836, 837 Myogenic heartbeat, 835 Myoglobin, 443-444, 445, 861 Myosin, 73, 289

in cytokinesis, 164

microfilaments and, 833 Myosin filaments

in fast-twitch muscle fibers, 841

in muscle contraction, 833-834, 836, 837-839

in myofibrils, 836, 837

structure of, 837 Myosin kinase, 839 Myosin phosphatase, 839 Myotonic dystrophy, 339 Myriapods (Myriapoda), 571, 578 Mytilus californianus, 987 Myxamoebas, 497-498 Myxomycota, 496-497 Myxomyosin, 497

NAD. See Nicotinamide adenine dinucleotide

NADH-Q reductase, 125-126,

127 NADP-reductase, 143 145 Naked-mole rats, 955 Nalgene, 495-496 Names. See Biological names nanos gene, 306 Nanos protein, 306, 307 Nasal cavity passages

olfaction and, 797-798

of whales, 752 Nasal salt glands, 912, 923 Nasopharyngeal cancer, 343 National Cancer Institute, 1043 Natural immunity, 360 Natural killer cells, 355 357 Natural selection, 2-3, 397

altruistic behavior and, 954-956

effects of, 404-406

reproductive value and, 969-970 Nature Conservancy, The, 1034 Nauplius, 571 Nautiloids, 562 Nautilus, 562

N. belazvnsis, 561 Navel orange trees, 665-666 Navigation

Incoordinate, 941, 943-944

by distance-and-direction, 941-943

piloting, 940 Neanderthals, 438, 598 Necrosis, 173 Necrotic lesions, 680 Nectar guides, 524 Negative feedback, 699

controlling hormone secretion, 721-722

in Cortisol regulation, 726

in regulation of hormone receptors, 729 Neher, Envin, 783 Neisseria gonorrhoeae, 749 Nematocysts, 548, 549, 846 Nematoda, 566, 574, 578 Nematodes

genome, 262

parasitic fungi and, 532

See also Caenorhabditis elegans; Roundworms Nematomorpha, 566, 574, 578 Nemertea, 556, 574, 578 Neoceradotus, 432 Neocortex, 818 Neomycin, 230 Neoteny, 411 Nephridiopores, 914, 925 Nephrons, 910

organization in the kidney, 918

structure and function, 915-916, 927 Nephrostomes, 914, 925 Neptune's necklace, 489 Nernst equation, 779 Neuroptera, 572, 574 Nerve cells

ion concentrations in and around, 89

sodium-potassium pumps, 89

See also Glial cells; Neurons

Nerve deafness, 804

Nerve growth factor (NGF), 714

Nerve impulses, 775, 777. See also

Action potentials Nerve nets, 774 Nerves, 815 Nervous systems

cell types in, 775-776 cerebral cortex, 819-821 components of, 814-815 development of, 816 digestive tract and, 903 generation and conduction of

nerve impulses, 776-785 glucose requirements of, 904 hormonal control of insect

molting and, 716 information flow in, 815 limbic system, 818-819 neuronal networks, 776, 792 overview of, 698, 773-775 peripheral system, 774, 776,

815, 816 processing visual information,

823-824 regulation of blood pressure,

882, 883-884 reticular system, 818 spinal cord anatomy and functioning, 817-818 synapses and neurotransmitters, 785-792 See also Autonomic nervous system; Brain; Central nervous system Nervous tissue, 695, 697, 698 Nesting, in eusocial animals, 955 Net primary production, 994-994 Net production, 996 Neural plate, 765 Neural tube

birth defects, 766 central nervous system development and, 816 formation of, 765-766 Neurohormones, 718, 815

Neuromuscular junction,
 785-786 Neuronal networks, 776, 792 autonomic nervous system,
 821-823 visual information processing, 823-824 Neurons, 697, 773
 action potentials, 780-783,
 784-785 anatomy, 775 depolarization and hyperpolarization, 779, 780 ion pumps and channels,
 777-778 long-term potentiation, 791,
 792 membrane and resting potentials, 776-777 neurohormones and, 718 neuronal networks, 776, 792 neurotransmitters and, 723,
 714 number in the brain, 814 patch clamping, 783
 summing of excitatory and
 inhibitory input, 787-788 γ -aminobutyric acid and neurotransmitters, 775-776, 785-types of 5 - . also Nerve cells
 T₁-N. crassa, 218-220 Neurotransmitter receptors consequences of multiple
 types, 789 in learning and memory.
 789-790 Uptake of, 788-789 Neurotransmitters, 723, 714, 775 actions of, 790 depolarization of motor end
 plate, 786 in neuromuscular junction,
 785 receptor-dependent activity,
 789 released by action potentials,
 786, 787 removal of, 791-792 Neurulation, 765-767 Neutral mutations, 408 Neutral theory of molecular evolution, 439-440
 Neutrons, 17, 18, 19 Neutrophils, 355, 357 Newborns, genetic screening,
 340-341 Newtons, 850
 New World monkeys, 596 New Zealand
 human-caused extinctions in,
 1030 vicariant distribution in, 1010 Niacin, 892 Nickel, 636 Nicotinamide, 892 Nicotinamide adenine dinucleotide (NAD)
 allosteric regulation of metabolism, 133 in citric acid cycle, 122, 223.
 124 electron carrier properties, 116 in fermentation, 129, 230 in glucose metabolism, 130 in glycolysis, 117, US, 119,
 120, 222 in pyruvate oxidation, 122
 123 reduced and oxidized forms,
 116 in respiratory chain, 125-126, 227 Nicotinamide adenine dinucleotide phosphate (NADP 137, 138, 143, 144, 147, 24\$
 Nicotinic acid, 892 Nicotinic receptors, 788, 789 Night blindness, 892 Night length, flowering and,
 672-673 Night vision, 809 Nirenberg, Marshall N., 224-225 Nitrate, 639, 642, 643 Nitrate reduction, 129, 642, 643
 Nitric acid, in acid precipitation,
 1004 Nitric oxide
 as neurotransmitter, 789, 790
 as second messenger, 289 Nitric oxide synthase. 289 Nitrification, 465-466, oil 643 Nitrifiers, 465-466 642
 1003-1004 Nitrite, 642, 643 Nitroreductase, 465-466 Nitrogen, 1003
 carnivorous plants and, 634
 in fertilizers, 639
 heavy, 207
 as nutrient, 634

in plant nutrition, 636 Nitrogenase, 640-641 Nitrogen cycle, 466, 642-643

1003-1004 Nitrogen deficiency (plant), 636 Nitrogen fixation, 465, 466

costs of, 1003

global nitrogen cycle and, 642-643

industrial, 641-642

nitrogenase in, 640-641

nitrogen fixers, 640

symbiotic associations in, 640, 641 Nitrogen fixers, 640, 642 Nitrogen metabolism, in

prokaryotes, 465-466 Nitrogenous bases, 47, 48

genetic code and, 224

in RNA, 220 Nitrogenous waste

excretion of, 912-914 also Excretory systems Nitroglycerin 285 Nitrosomonas, 465-466 Nitrous oxide, 236, 237
NMDA receptor, 789, 790, 791 Nodes of Ranvier, 784-785 Nodes (plant), 604, 611 Nod factors, 641 Nodules, in roots, 640,
641, 642 Noller, Ham., 229 Nomarski optics, 57 Noncoding DNA, 446-447 Noncompetitive inhibitors, 109 Noncovalent
bonds, 214 Noncyclic electron flow, 143-144 Nondisjunctions, 172, 194, 231 Nonidentical twins 743 Nonpolar interactions, 24
—25 Nonrandom mating, 404 Non-REM sleep 825-826 Nonseed tracheophytes

characteristics of, 505-508

club mosses, 511-512

early forms of, 505-509

evolution of, 508, 510-511

ferns, 513-515

homosporous and heterosporous in, 511

horsetails, 512

leaves, 510-511

phyla of, 501

roots, 510

whisk ferns, 512-513

Nonsense mutations, 2 Nonspecific defenses. See Innate

defenses Nonspontaneous reactions, 99 Nonsynonymous substitutions,

441

Nontracheophytes

characteristics of, 502, 503

defined, 501

hornworts, 505-506

life cycle, 504

liverworts, 504-505

mosses, 506-507

phyla of, 501 Noradrenaline. See

Norepinephrine Norepinephrine, 725

autonomic nervous system and, 821, 822

heart pacemaker cells and, 873

as neurotransmitter, 789, 790

smooth muscle contraction and, 834, 835

targets and actions of, 739 Normal flora, 356 Norplant, 746 North America, human-caused extinctions in, 1030 Northern elephant seals, 403 North Star, 942 Nosocomial infections, 239 Nothofagus, 1017 Notochord in chordates, 582 in evolution, 430, 433 formation of, 765 in vertebrates, 583 Nuclear envelope, 62, 63, 260 during cell division, 64 during mitosis, 161, 362, 163, 164 Nuclear lamina, 62, 63-64 Nuclear matrix, 63 Nuclear pores, 62, 63, 270 Nuclear transplant experiments, 297 Nuclease, 903 Nucleic acid hybridization, 265-266 Nucleic acids base sequence uniqueness, 48 structures and properties, 47-18 types of, 47 See also DNA; RNA Nucleoid, 58 Nucleolus/Nucleoli, 62, 63 in animal cells, 60 during mitosis, 161, 164 in plant cells, 63 Nucleoplasm, 62, 63 Nucleosides, 47 Nucleoside triphosphates, 214, 235 Nucleosomes, 160, 363, 261, 273-274 Nucleotide bases, tautomeric forms, 236, 237 Nucleotides biological roles of, 49 components of, 47 in DNA, 203, 204, 205, 208 phosphodiester linkages, 47-48 prokaryote evolution and, 467-468 Nucleotide substitutions rates of, 441 synonymous and nonsynonymous, 438 Nucleus in animal cells, 60 during mitosis, 161, 362, 163, 164 multiple, in ciliates, 485-486, 487 in plant cells, 63 structure and function, 62, 63-64 Nucleus (neurons), 818 Null hypothesis, 12 Nurse cells, 305 Nurse plants, 959, 961 Nutrient deficiency diseases, 892-893 Nutrients acquisition of, 634-635 essential elements for plants, 635-637 overview of, 634 See also Macronutrients; Micronutrients; Mineral nutrients Nutrition. See Animal nutrition; Plant nutrition Oaks, gypsy moths and, 989

Obesity, 886, 906

Obligate aerobes, 464

Obligate anaerobes, 464

Obligate intracellular parasites, 240

Obligate parasites, 532

ob/ob mutation, 906

Occipital lobe (cortex), 819, 821, 823-824

Oceans

currents, 993, 3027 cycling of materials in, 999 dispersal of marine organism, 1026-1027 hydrological cycle, 1001 influences on climate, 993-994 osmolarity of, 911 zones of, 1026, 3027

Ocotillo, 686

Octet rule, 20

Octopus cyanea, 561

Octopuses, 563, 562, 841-842

Ocyurus chrysurus, 586, 590

Odonata, 572

Odontochile rugosa, 568

Odorant molecules, 290-291

Odorants, 798

Oedogonium, 492

Off-center receptive fields, 810

Oils, 49-51

Okazaki, Reiji., 211

Okazaki fragments, 211-212

Old World monkeys, 596

Oleic acid, 50

Olfaction, 797-798

in dogs, 794, 798

in snakes, 799 Olfactory bulb, 797, 798 Olfactory organs, 290-291 Oligochaetes (Oligochaeta), 558, 578 Oligodendrocytes, 776 Oligo dT, 319

Oligosaccharides, 43, 44, 53, 82 Oligosaccharins, 647, 660 Omasum, 902, 903 Ommatidia, 807 Omnivores, 893, 995, 996

teeth, 894, 895 ompF gene, 282 OmpF protein, 281, 282 OmpR protein, 281-282 Onagers, 3009

On-center receptive fields, 810 Oncogenes, 275, 344, 345, 346 Oncomelania, 425, 426 One gene, one polypeptide hypothesis, 218-220 Onorhynchus, 968, 969 Onychophorans (Onychophora), 568, 578 Oocytes, 735, 736 Oogenesis, 735, 736 Oogonia, 735, 736 Oomycetes, 490⁹¹ Oomycota, 477 Ootids, 735, 736 Oparin, Alexander, 453 Open circulatory systems, 867 Open reading frames, 255 Open systems, 97, 98-99 Operator-repressor systems

inducing transcription, 251-252

repressing transcription, 252-253 Operators, 251-253

in viruses, 254, 255 Opercular flaps, 853, 854 Operons

lac operon, 251-252, 254

trp operon, 252-253 Ophiuroidea, 578, 581 Opiothrix suemsonii, 580 Opium poppies, 907 Opossums, 594 Opportunistic infections, 373 Opportunity costs, 948 Opposite birds, 591 Opsin, 805, 809 Optical isomers, 32 Optic chiasm, 824 Optic cup, 302 Optic nerve, 809, 823, 824 Optic vesicle, 301 Optimality modeling, 949-950 Opuntia, 971-972 Oral cavity, 897 Oral contraceptives, 746, 747 Orange trees, 665-666 Orangutans, 433, 596, 597 Orchids, 523, 985 Orcinus orca, 752 Orconectes palmeri, 570 Orders, 434 Ordovician period, 380-381, 387

Organelles, 5

cytoplasmic inheritance, 196

endomembrane system, 64-67

endosymbiosis and, 70, 479

energy processing, 67-71

information processing, 63-64

mutation levels, 196

peroxisomes, 71

in phylogenetic studies, 431

plastids, 68-70

targeting polypeptides to, 231, 232

types and functions of, 62-63

vacuoles, 71-72 Organic fertilizers, 639 Organic molecules, 31 Organic phosphates, 33 Organ identity genes, 304-305

in flowering, 671 Organisms, 8 Organ of Corti, 803, 804 Organogenesis, 765-767 Organs, 8

tissues in, 695, 697-698 Organ systems

defined, 698

mammalian, 698 Orgasm, 745

Orientation, in animal navigation, 940-944 Origin of life, 450-451

guiding principles for research in, 451

necessary conditions for, 451-452

protobionts, 452-454 Origin of replication, 209, 230

DNA vectors and, 315 Origin of the Species, The

(Darwin), 397 Orthoptera, 572 *Oryza sativa*, 167 Oscula, 547

Osmoconformers, 911, 912 Osmolarity, 911 Osmoregulators, 911, 932 Osmosensors, 923 Osmosis, 86-87, 621 Osmotic potential (pressure), 46

capillaries and, 876-877 *Osmunda cinnamomcea*, 166 Ospreys, 907 Ossicles, 802, 803, 804 Ossification, 844 Osteichthyes, 578, 585 Osteoblasts, 723, 724, 843, 844 Osteoclasts, 723, 724, 844 Osteocytes, 843, 845 Ostia, 867 Ostracoderms, 583 Ostrich, 448 Otoliths, 802 Outcrossing, 432 Outer membrane, bacterial, 59 Outgroups, 428 Ova

blocks to polyspermy, 754, 755

cytoplasmic rearrangements, 755-756

egg-sperm recognition mechanisms, 753-754

fertilization, 741-742

formation of, 735, 736

nonmotility of, 734

ovarian cycle and, 742-743

release from ovary, 741

See also Eggs Oval window, 802, 803 Ovarian cycle

hormonal control of, 743-745

overview of, 742-743 Ovary (animal), 724, 734, 741

follicles, 742, 743-744, 745

hormones of, 729

ovarian cycle, 742-745 Ovary (plant), 522, 522, 666 Overdominance, 189 Overexploitation, 1038

of whales, 970, 972 Overnourishment, 889 Oviducts, 741, 742

gamete intrafallopian transfer, 749

tubal ligation, 747 Oviparity, 738-739 Ovoviviparity, 739 Ovulation, 742-743

contraceptive methods and, 746 Ovules, 666

angiosperm, 521-522

gymnosperm, 519 Oxalic acid, 689 Oxaloacetate, 109, 223, 124, 150,

151 Oxidation, 115

Oxidation-reduction reactions, 26. See also Redox reactions Oxidative phosphorylation,

125-129 Oxidizing agents, 115 Oxidizing atmosphere, 451 *Oxycomanthus bennetti*, 580 Oxygen

effects on breathing rate, 864

evolution of life and, 380, 382, 451-452, 454-455, 466

as limiting resource, 975

myoglobin and, 861

as nutrient, 634

in ocean waters, 999

oxidation of NADH, 116

oxygen-hemoglobin binding properties, 860-862

ozone layer and, 4

partial pressures, 851

photorespiration and, 148-149

in photosynthesis, 137

physical properties of gas exchange, 849-852

in water-saturated soils, 687, 688 Oxytocin, 717-718, 719, 770 Ozone, 4,1000

Pacemaker cells, 821, 835, 873,

874 Pacific salmon, 968, 969 Pacific yew, 1043 Pacinian corpuscle, 800 Packed-cell volume (blood), 879

Pair rule genes, 306, 307 Paleomagnetism, 381 Paleozoic era, 380-382, 387-389 Palisade mesophyll, 618 *Palmaria palmata*, 491 Palm trees, 527, 617 Palolo worms, 732 Palo verde tree, 959 Pancreas, 724, 897, 901

digestion and, 899, 900

hormones of, 728

insulin and, 904

secretin and, 903 Pancreatic amylase, 902 Pangaea, 380, 383-384, 388-389,

508, 1008 *Pan paniscus*, 596 *Panthera tigris*, 935 Panting, 707 Pantothenic acid, 892 *Papaver somniferum*, 907 *Papilio memnon*, 573 *Papillae*, 798

Papillomaviruses, 277, 343 *Papio*, 957 Pap smears, 695 Parabronchi, 855 Paracina, 486

Paracrine hormones, 713-714 Paracrine signals, 280 Parallel evolution, 427 Paralysis, 818 *Paramecium*

anatomy, 486

food vacuoles, 481

motility in, 486, 487

P. bursaria, 486

P. caudatum, 481

reproduction and conjugation in, 486-187 Parapatric speciation, 418 Paraphyletic groups, 435, 436 Parapodia, 558 Parasite-host interactions,

978-979 Parasites, 978

defined, 974

flatworms, 554

fungi, 532

leeches, 559

plants, 643-644

roundworms, 567

wasps, 976, 977 Parasympathetic nervous system, 821-823, 873, 883 Parathormone. See Parathyroid hormone
Parathyroid glands, 724, 728, 723, 724 Parathyroid hormone, 728, 723, 724 Parenchyma, 608, 609
leaf mesophyll, 618
transfer cells, 623-624
in vascular rays, 615-616 Parental generation (P), 179 Parenting behavior, 956
number of offspring and, 968 Paresthesia, 818 Parietal lobe, 819, 820
Parks
economic lands and, 1042 priorities for locating, 1040, 1042
Parotid salivary gland, 897
Parrots, 592
Parsimony principle, 430, 1010
Parthenogenesis, 733-734
Partial pressure
of carbon dioxide, 851-852 of oxygen, 851
Particle bombardment, 317
Particulate theory of inheritance, 180
Parturition, 770-771
Parulidae, 435
Passenger pigeons, 1038
Passeriformes, 435
Passive immunization, 364
Passive transport, 85-88, 89
Pasteur, Louis, 114, 455, 456
Pastoralism, 599
Patch clamping, 783
Patella, 843
Pathogenesis-related proteins, 680, 681
Pathogens
bacterial, 466-467, 471-472 fungal, 533
kinetoplastids, 483, 484 plant-pathogen interactions, 679-681
Pattern formation apoptosis in, 302-304 body segmentation in
Drosophila, 305-308 in development, 295 morphogenesis and, 302 organ identity genes, 304-305 positional information, 305
Pattern formation genes, 671
Pauling, Linus, 203
Pavlov, Ivan, 827
Pax genes, 309, 766-767

PCBs. See Polychlorinated biphenyls

PCR. See Polymerase chain reaction

Peanut oil, 51

Pears, 525

Peas, in Mendel's experiments, 277, 178-180, 181-182

Peat, 500

Pecking response, 928

Pectinatella magnifica, 960

Pectoral girdle, 843

Pedigree analyses, 284, 185

Pedigrees, 185

Pelagic zone, 1026, 2027

Pelamis platurus, 1027

Pellagra, 892

Pellicles, 482, 486

Pelmatozoa, 580-581

Pelvic girdle, 843

Pelvic inflammatory disease, 748-749

Penetrance, 189

Penguins, 592, 738

Penicillin, 230

Penicillium, 536, 988

Penile circumcision, 176

Peninsulas, species richness in,

1014 Penis, 737

in sexual excitement, 745

urethra and, 740 Pennisetum setaceum, 522 Pentoses, 43, 47-48 Penumatophores, 687 PEP carboxylase, 150, 151 Peppers, 974 Pepsin, 111, 898, 899, 901 Pepsinogen, 898, 899 Peptidases, 896 Peptide backbone, 38, 39 Peptide linkages, 37-38, 229 Peptide neurotransmitters, 785,

789, 790 Peptidoglycan, 59, 463 Peptidyl transferase activity, 229 Perdeck, A. C, 927-928 Peregrine falcon, 7, 1040

Perennial plants, 648, 671 Perfect flowers, 522 Perforin, 368 Perfusion, 853 per gene, 933, 939 Pericycle, 614 Periderm, 613 Perilla, 675 Period

of circadian rhythms, 937

of cycles, 673 Periodic table, 28 Peripatodes novaezealandiae, 568 Peripheral membrane proteins,

80,82 Peripheral nervous system, 774

development of, 816

divisions of, 815

information flow and, 815

Schwann cells in, 776 Periplasmic space, 463-464 Perissodus microlepis, 409 Peristalsis, 897-898 Peritoneum, 544, 545, 897 Peritubular capillaries, 915, 916,

927, 920 Permafrost, 1016 Permeable membranes, 85-86 Permian period, 508

climate of, 383
 evolutionary trends in, 388-389
 fauna of, 392
 major events in, 380-381
 plant evolution in, 518 Pernicious anemia, 892, 893 Peroxisomes, 71, 149
 in animal cells, 60
 in plant cells, 62 Per protein, 939 Pesticides, bioaccumulation of,
 907 Pest management
 integrated pest management, 998-999
 life histories and, 970-972 Pests, introduced, 1037 PET. See Positron emission
 tomography Petals, 522, 522, 666, 672
 Petio
 . u-lilioi pyrrhonota, 4(h> Petromyzon marinus ~> s I
 I ene ; 4 _> pH
 buffers and, 30
 defined, 29 30
 effect on enz) mes, 111, 112 !so Blood pH; Soil pi 1 Phaeophyta, 477 Phage. See Bacteriophage Phagocj tes J58 479
 amoeboid mo\ ement in, 833
 in lymph nodes. 877
 o\ en iew ot. 354
 types ot. \$57 Phagocytosis, 66-67, 91, 357, 364 Phagosomes, 67 Phalanges 843 Phalaris canariensis, 653 Phantom limbs, 795
 Pharamacogenomics, 350 Pharyngeal basket, 582, 583 Phar\ngeal slits, 581, 582 Pharynx, 857, 858, 897, 898
 in hemichordates, 581, 582
 in roundworms, 566 Phase-advanced rhythms, 937 Phase contrast microscopy, 57 Phase-delayed rhythms, 937 Phaseolus
 vulgaris, 684 Phases, of circadian rhythms, 937 Phase shifts, 674 Phelloderm, 617 Phenolics, 683 Phenotype, 180
 defined, 398
 fitness and, 398
 phenotypic variation, 189
 plasticity, 410
 recombinant, 182 Phenotypic plasticity, 410 Phenylalanine, 37, 224, 340, 346,
 ' 889, 890 Phenylalanine hydroxylase, 332,
 ' 340,346 Phenylketonuria (PKU)
 comparison with sickle-cell anemia, 338
 discovery of, 331
 enzyme failure in, 332
 inheritance of, 335
 screening for, 340
 treatment of, 346 Pheromones, 534, 713, 797, 847,
 935 Philadina roseola, 555 Phillips, David, 106 Phinney, Bernard O., 651-652 Phloem, 502-503, 608, 610-611

in roots, 624

secondary, 615, 626, 617

translocation in, 628-632

transport in, 621

in vascular bundles, 615

vascular cambium and, 613 Phlox, 432

Phoenix dactylifera, 527 Phoca vitulina, 866 Phoronids (Phoronida), 555-556, 574, 578

Phosphate, in lake eutrophication. 1004-1005

Phosphate group, 31 Phosphatidylinositol (PIT), 287-288

signaling system, 754 Phosphodiesterase, 806, 807 Phosphodiester linkages, 47-48,

204, 214 Phosphoenolpyruvate, 121, 150 Phosphofructokinase, 119, 220,

133 2-Phosphoglycerate, 222 Phosphoglycerate kinase, 120,

222' 3-Phosphoglycerate (3PG), 222,

147, 248, 150, 152 Phosphoglyceromutase (2PG),

222 Phosphohexose isomerase, 220 Phospholipases, 287, 288 Phospholipid bilayers, 51, 79-81.

See also Membranes Phospholipids

hydrophilic and hydrophobic

properties, 80 in membranes, 51, 79-81 as second messengers, 287-288 Phosphorus

in animal nutrition, 890 in fertilizers, 639 as nutrient, 634 in plant nutrition, 636 Phosphorus cycle, 1004-1005 Phosphorus deficiency (plant),

636 Phosphorylase, 287 Phosphorylase kinase, 291 Phosphorylation, 158

protein kinases receptors and,

283 of proteins, 233 Photoautotrophs, 464, 470 Photoexcited rhodopsin, 806 Photoheterotrophs, 464, 465 Photons, 138

absorption by pigments,

139-140 See also Light Photoperiodicity, 727, 940 Photoperiodism

biological clock and, 673-674,

940 day length and night length

in, 671-675 flowering hormone and,

674-675 photoreceptors and, 673 Photophosphorylation, 138, 142,

145-146 Photoreceptors, 795

basis of photosensitivity in,

805-807 blue-light receptors, 661,

662-663 phytochromes, 661-662 in plant development, 647, 648 in retina structure and function, 809-812 rod cell anatomy, 80 Photorespiration, 148-149

Photosensitivity, 805-807 Photosensors, 795 Photosynthesis

accessory pigments in, 141

action spectrum, 140-141

Calvin-Benson cycle, 146-148

C₃ and C₄ plants compared, 249, 150-151

chlorophylls in, 141

crassulacean acid metabolism, 151

creation of atmospheric oxygen and, 454-455, 466

emergence and evolution of, 4, 146

formula for, 137

identifying reactants and products of, 136-137

laws of thermodynamics and, 136

leaf anatomy and, 617-618

light reactions, 142-146

measures of productivity, 994-995

overview of, 137-138

photorespiration, 148-149 Photosynthesizers, 635, 995, 996 Photosynthetic bacteria, 59

cyanobacteria, 470

photoautotrophs, 464

photoheterotrophs, 464, 465

proteobacteria, 469

size range, 240 Photosynthetic carbon reduction cycle. See Calvin-Benson cycle Photosystems

defined, 138

system I, 243, 144, 245

system II, 243, 144, 245 Phototropin, 662, 663 Phototropism, 653, 654 Phthiraptera, 574 Phycobilins, 141 Phycocyanin, 240, 491 Phycoerythrin, 240, 491 Phycomyces, 534 Phyla. See Phylum/Phyla Phyllopteryx taeniolatus, 586 Phylloxera, 677

Phylogenetic trees

consensus trees, 430

described, 425-426

how to read, 426

maximum likelihood method, 430

molecular traits, 430-431

morphological traits, 430

parsimony principle, 430

reconstructing, 426-430

uses of, 431-432 Phylogeny

classification and, 435¹³⁶

defined, 425

reconstructing relationships, 426-430

using in biogeography, 1008, 2009 Phylum/Phyla, 434 Physarum, 497 Physical map markers, 349 Phytoalexins, 680

Phytochromes, 291-292, 661-662

biological clock and, 674

photoperiodism and, 673 Phytophthora infestans, 491 Picture-winged fruit fly, 418 Pigeons, 953

artificial selection and, 397

crop milk, 714

homing in, 940, 943 Pigmented epithelium, 809, 810 Pigments

absorption of photons, 139-140

absorption spectrum, 140

accessory, 141

antenna systems, 142

B-carotene, 51-52

chlorophylls, 141

fluorescence, 142

ground and excited states, 139-140, 142 Pileus, 538, 539 Piloting, 940 Pilus/Pili, 59, 246 Pima Indians, 350 Pineal gland, 724, 729, 938 Pineapples, 525

Pine needles, decomposition, 988 Pine trees, 519-520 Pin flowers, 404 Pink molds, 536 Pinocytosis, 91 Pinus

P. aristata, 603

P. sylvestris, 988 Piper nigrum, 974 Pisaster ochraceous, 986-987 Pisolithus tinctorius, 540 Pistil, 521, 666 Pisum sativum, 177 Pitcher plants, 644 Pith

in roots, 614

in stems, 615 Pith rays, 615 Pithys albifrons, 1036 Pit organs, 812 Pits, 608, 609

Pituitary dwarfism, 719-720 Pituitary gland, 724, 923

anterior, 717, 719-720

development, 717

hypothalamus and, 717, 720-721

posterior, 717-719

regulation of blood pressure and, 882 Pit vipers, 812 Pivotal joint, 845 Pivots, 845

PKA (protein kinase A), 290, 291 PKC (protein kinase C), 288, 289 Placental mammals, 595 Placentas, 589, 742, 768 Placoderms (Placodermi), 584,

585 Placodonts, 393 Plague, 467 Plaice, 410 Planaria, 867, 914. See also

Flatworms Planarians, 554

Planck, Max, 139 Planck's constant, 139 Plane joint, 845 Plankton, 496 Plantae, 9, 10, 434, 500-502 Plant-animal mutualisms,

984-985 Plant biogeography, 1011 Plant biotechnology

improved nutritional characteristics of crops, 327 plant expression of insecticides, 325-326, 684 plant resistance to herbicides,

327 public concerns with, 327-328 Ti plasmid vector, 316 Plant breeding

contributions to genetics,

176-177 controlled crosses, 177 dihybrid crosses, 182 Mendel's experiments,

178-180, 181-182 monohybrid crosses, 178,

179-180 reciprocal crosses, 177 test crosses, 181-182 Plant cells

cloning of, 297 cytokinesis, 164, 265 distinguishing characteristics,

607 growth in, 657 microtubules in, 74 plasmodesmata, 292-293, 632 structure and organization, 61 totipotency in, 297 as transgene hosts, 315 turgor pressure, 87, 621 types, 608-610 Plant cell walls, 61 auxin and, 657

cellulose microfibrils, 656-657 plant growth and, 657 structure and function, 76,

607-608 turgor pressure and, 87 Plant classification, 434 Plant communities amensalisms in, 983 animal effects on composition

and structure, 986, 987 See also Biomes Plant defenses

biotechnology and, 684 defensive compounds, 680 gene-for-gene resistance, 681 hypersensitive response, 680,

681 plant hormones in, 660 plant mechanical barriers, 679 plant protections from secondary compounds, 684-685 role of signaling in, 683-684 secondary compounds,

682-683, 982 systemic acquired resistance, 680-681 Plant development

characteristics of, 611-612

embryo development in seeds, 668-670

flowering, 648

hormones in, 650-661

interacting factors in, 646-647, 655-656, 658

light and, 661-663

meristems in, 612-613

morphogenesis in, 295

organ identity genes, 304-305

overview of, 647-649

patterns in, 611

root tissues, 613-614

secondary growth, 615-617

seed germination, 649-650, 651

senescence, 648-649

shoot development patterns, 648

stages of, 295

stem tissues, 614-615 Plant diseases

crown gall, 469

fungal, 533, 536 Plant embryo, 668-670

heart-shaped, 669

torpedo stage, 669 Plant hormones

abscisic acid, 660

auxins, 652-658

brassinosteroids, 660-661

cytokinins, 658

ethylene, 658-660

gibberellins, 650-652

interactions between, 655-656, 658

in plant defenses, 660

in plant development, 646-647, 648

salicylic acid, 681

typical activities of, 647 Plant kingdom. See *Plantae* Plant litter decomposition, 988

humus, 639-640 Plant nutrition

acquisition of nutrients, 634-635

carnivorous plants, 634, 644

essential elements, 635-637

ion exchange, 639

nitrogen fixation, 640-643

nitrogen metabolism, 642

parasitic plants, 643-644

soils and, 637-640

sulfur metabolism, 643 Plant-pathogen interactions, 679-681 Plant-pollinator interactions

coevolution, 523-524, 985-986

mutualisms, 984, 985 Plant reproduction

asexual, 676-677

flowering, 670-676

overview of, 665-666

sexual, 666-670 Plants

adaptations to life on land, 502-503

alternation of generations, 167, 489⁹⁰, 501

antitranspirants, 628

apoplast and symplast, 623-624 associative mating in, 404 calcium-induced calcium release in, 289 C₃ and C₄ plants compared, 249, 150-151 classification of, 502 coevolution, 985-986 competitive interactions, 976, 977 costs of herbicide resistance, 407 crassulacean acid metabolism, 628 determinate and indeterminate growth in, 612, 613, 670, 671 drought stress and gene expression, 273 in dry environments, 685-687 effects on soil, 639-640 fires and, 516 genetic variation in, 399 growth as locomotion, 635 in habitats with heavy metals, 689 herbivores and, 681-685 hydroponic culture, 635 measures of photosynthetic productivity, 994-995 metabolic pathways, 151-152 mutualism, 983, 984-985 mycorrhizae and, 532, 539-540, 614, 641, 983 nitrogen cycle and, 642-643 nontracheophytes, 501, 502, 503-507 nutrient deficiency symptoms, 635-636 parapatric speciation in, 418 parasitic fungi and, 531, 532 phloem translocation, 628-632 phyla, 501 polyploidy in, 417 as primary producers, 995, 996 protein kinase receptors in, 283 responses to light, 280 responses to pathogens, 679-681 in saline environments,

688-689 sexual reproduction in, 167 subpopulations, 408-409 survivorship curves, 963 temperature extremes and, 689-690 transpiration, 626-628 uptake and transport of water and minerals, 620-626 vascular tissue, 502-503 in water-saturated soils, 687, 688 See also Flowering plants;

Gymnosperms;

Tracheophytes Plant succession, 987-988 Plant tissue culture agricultural applications, 677 plant hormones and, 658 Plant vacuoles, 62 in cell growth, 657 functions of, 67, 71 in halophytes, 688-689 in plant protection from secondary compounds, 684 in xerophytes, 687 Plant viruses plasmodesmata, 293 transmission of, 243-244

Planula, 549

Plaque, atherosclerotic, 878-879

Plasma, 86, 879, 880, 881-882

Plasma cells, 355, 359, 362-363, 367

Plasmagel, 833

Plasma membrane in cell evolution, 478-479 electric currents and, 779 in plant cells, 62 in plasmodesmata, 292 in prokaryotes, 58, 59, 67, 462 refractory period, 780 structure and function, 58 See also Membranes

Plasmasol, 833

Plasmids transposable elements and, 249 types of, 248-249 as vectors, 315, 316

Plasmin, 324

Plasminogen, 324

Plasmodesmata, 62, 76, 292-293, 607-608, 632

Plasmodium, 485

Plasmodium, 497

Plasmogamy, 532

Plasocytes, 355

Plastids chloroplasts, 68-69 chromoplasts, 69, 70 cytoplasmic inheritance, 196 leucoplasts, 70 Plastocyanin, 145

Plastoquinone, 145

Platanus, 418

Platelet-derived growth factor, 159, 323

Platelets, 159, 354, 355, 880-881

Platyhelminthes, 553-554, 574, 578

Platypiza crassirostris, 416

Plecoptera, 572

Plectorhinchus chaetodonoides, 586

Pleiotropy, 187-188

Pleistocene epoch, 390, 392

Pleural cavities, 858, 859

Pleural membranes, 858

Pluripotent hematopoietic cells, 355

Pneumococcus, 200-201

Pneumocystis carinii, 373, 533

Pneumonia, 55, 533 in AIDS, 373

Podocytes, 916, 927

Poikilotherms, 701

Point mutations, 234-235, 338

Polar bears, 887

Polar bodies, 735, 736

Polar covalent bonds, 23

Polarity

in cell determination, 300-301 maternal effect genes and, 305-306

Polar microtubules 161, /<>2 163 Polar molecules! 23, 24 - > Polar nuclei, 666 667 Polio 360

Polio\ iru 240

Pollen cones

Pollen grains, 517 U8

development of 666 667

methods of transport, 667-668

in self-incompatibility, 668

tube cell and generate e cell in. 668 Pollen tubes, -17.668 Pollination, 667-668 Pollinators

coevolution and, 523-524

mutualisms, 984, 985 Pol) A tail, 269, 319 Polychaetes (Polychaeta), 558, 578, 732 Pol\chlorinated biphenyls

(PCBs), 908 Polyclonal antibodies, 364 Polygenes, 189 Polymerase chain reaction (PCR), 214, 216-217

diagnosing infections with, 329

in DNA testing, 341

in molecular evolution studies, 440 Polymers

condensation and hydrolysis of, 35

defined, 34

origin of life and, 452

simple and complex, 35

See also Macromolecules Polymorphic alleles, 186 Polymorphisms

in populations, 409

in proteins, 332 Polynucleotides

directionality in, 204

See also DNA; RNA Polypeptides

chains, 36, 37-38, 39

formation during translation, 228-229

noncovalent interactions, 41

one gene, one polypeptide hypothesis, 218-220

posttranslational modifications, 233

posttranslational targeting, 231-233

signal sequences, 231

See also Proteins Polyphyletic groups, 435 Polyplacophora, 560, 578 Polyploidy, 443

and cell division, 173

in sympatric speciation, 417 Polyproteins, 233 Polyps, 548, 549-551 Polyribosomes, 230-231 Polysaccharides

biological functions, 45-46

catabolic interconversion, 131

defined, 43

osmotic pressure and, 46

in plant cell walls, 679

Polysiphonia, 41 Polysomes, 230-231 Polyspermy, blocks to, 754, 755 Polyterpenes, 683 Polyunsaturated fatty acids, 50 Pongopygmaeus, 596 Ponds, 816, 818 Population density

defined, 959

effects of disturbances on, 966

influence on birth and death rates, 965-966

measuring, 960-961 Population dynamics, 962-963 Population ecology

life histories, 967-970

population dynamics, 962-963

population growth, 963-965

population regulation, 965-967

population structure, 959-962 Population genetics

allele frequencies, 399-400

associative mating, 404

fitness, 398

gene flow, 402
 gene pools, 398
 germ-line mutations, 402
 Hardy-Weinberg equilibrium, 400-402
 mechanisms maintaining
 genetic variation, 408-409
 natural selection, 404-406
 polymorphisms, 409
 random genetic drift, 402-404 Population growth
 carrying capacity and, 964
 exponential, 963-965
 human, 600, 970-972
 life histories and, 967-970
 logistic growth, 964-965
 under optimal conditions, 965
 rescue effects, 965 Population management,
 970-972 Populations, 8
 bottlenecks, 402-403
 defined, 959
 human management of, 970-972
 modeling, 1031-1032 Population structure
 age distributions, 961-962
 density, 959-961
 spacing patterns, 960, 961 Popuhis trcimtlodes, 960 Pore complex, 62, 63 Porifera, 547, 578 Portal blood vessels, 721
 Portuguese man-of-war, 548, 846 Positional cloning, 336-337 Positional information, 305 Positive cooperativity, 860 Positive
 feedback, 699 Positron emission tomography
 (PET), 829 Postabsorptive period, 904, 905 Postelsia palmaeformis, 489 Postganglionic neurons, 821 Postsynaptic cells, 785
 long-term potentiation, 791, 792
 Postsynaptic membrane, 785
 gap junctions, 789
 integration of synaptic input, 786
 summing of excitatory and inhibitory input, 787-788 Posttranslational events, 231-233 Postzygotic reproductive barriers,
 419 Potassium
 in animal nutrition, 890
 in fertilizers, 639
 membrane potential and, 777
 in plant nutrition, 636
 in soils, 638 Potassium-40, 381 Potassium channels
 in action potentials, 780, 781, 782
 in depolarization and hyper-polarization, 780

in non-REM sleep, 826
 in pacemaker cells, 873
 in resting potentials, 778 Potassium deficiency (plant), 636 Potassium equilibrium potential, 778, 779 Potato blight, 490 Potatoes, 70, 167, 605, 606 Potential energy, 26, 96 Powdery mildews, 477, 536 Power arms, 845-846 Poxviruses, 240 p21 protein, 159 p53 protein, 159, 277 Prader-Willi syndrome, 339 Prairies, 986, 987 Prebiotic systems, 452-454 Precambrian period, 380-381, 382, 386-387 Precapillary sphincters, 882 Precipitation
 annual patterns, 1014
 latitudinal variation, 2015 Predation
 in evolution, 393
 fungi and, 532
 in protostome evolution, 575 Predator-host interactions, 974 Predator-prey interactions, 974, 979-982 Predators, 978
 defined, 974
 effects on community structure, 986-987
 introduced, 1037
 optimality modeling of foraging behavior, 949-950
 sea stars, 581 Preganglionic neurons, 821, 822 Pregnancy
 first trimester, 769-770
 labor and delivery, 770-771
 second trimester, 770
 spontaneous termination, 173
 third trimester, 770 Pregnancy tests, 364 Premature infants, respiratory distress syndrome, 858 Premolars, 894, 895 Pre-mRNA, 261
 alternate splicing, 276
 introns, 265
 processing of, 268-270 Prenatal testing, 340, 341, 768, 769 Pressure bombs, 626 Pressure flow model, 630-632 Pressure potential, 621 Presynaptic cell, 785 Presynaptic excitation, 788 Presynaptic inhibition, 788 Presynaptic membrane, 785, 789 Prey, 974
 defenses of, 980-982
 See also Predator-prey interactions Prezygotic reproductive barriers, 419 Priapulids (Priapulida) 565 Primaquine, 234 Primary active transport, 89, 90 Primary carnivores, 995, 996 Primary embryonic organizer, 762-763 Primary growth
 apical meristems and, 613
 in roots, 613-614
 in stems, 614-615 Primary immune response, 360 Primary lysosomes, 67 Primary meristems, 612-613 Primary mesenchyme, 759-760 Primary motor cortex, 820 Primary oocytes, 735, 735, 736, 742 Primary plant body, 612-615 Primary producers, 995, 996 Primary products, 682 Primary protein structure, 38, 39 Primary sex determination, 194 Primary somatosensory cortex, 820 Primary spermatocytes, 735, 740 Primary succession, 987-988 Primary walls, 607 Primary xylem, 615 Primase, 209, 211, 212 Primates

characteristic traits, 595

feeding ecology and social organization, 957-958

major lineages, 595-597

phylogeny, 595 Primer strands, 210-211, 222 Primitive groove, 764, 765 Primitive streak, 764 Primosomes, 211 Primrose, 404 Primula, 404 Prions, 334 Probability calculations

addition, 184

dihybrid crosses, 184-185

monohybrid crosses, 184

multiplication, 183-184 Proboscis

in acorn worms, 581, 582

in ribbon worms, 557-558 Procambium, 613 Procedural memory, 828 Progesterones, 726, 744, 745

oral contraceptives and, 746, 747

in pregnancy and labor,

769-770 structure of, 725 targets and actions of, 719

Progestins, 746

Programmed cell death, 173-174, 302-304. See also Apoptosis

Prokaryotes

beneficial roles of, 466 cell and cell wall characteristics, 462, 463-464 characteristic shapes and

forms, 461 conjugation in, 245-246, 247 DNA distribution, 156-157 DNA replication, 156 in element cycling, 466 emergence of, 3-4 endosymbiosis and, 70 energy transformation in, 67 evolutionary relationships,

467-168 fission, 155-157, 462 flagella, 75(n), 463-464 gene expression in, 221,

249-254 general biology of, 58, 461—166 generation times, 464 genome, 255-257, 260, 262 glucose metabolism in, 117,

118 lateral gene transfer in, 468 metabolic types, 464-466 motility in, 75(n), 462-463 mutations and, 468 pathogenic, 466-467 plasma membrane in, 67, 462 plasmids in, 248-249 promoters in, 271 reproduction in, 245, 464 reproductive signals, 156 ribosomes in, 225 signal transduction in,

281-282 size range, 58, 240 specialized structures in,

58-59 spores, 5

transcription in, 223 transduction in, 247 transformation in, 247 as transgene hosts, 315 translation in, 223 transposable elements in, 249 using in genetic research,

239-240 See also Bacteria

Prolactin, 714, 718, 719, 720

Prolactin release-inhibiting hormone, 721

Prolactin-releasing hormone, 721

Proline

genetic code for, 224

in plant vacuoles, 687, 688-689

side chain, 37

Prometaphase meiosis, 168, 171 mitosis, 162, 163-164

Promoters, 222, 230, 265

controlling adjacent genes, 251

controlling efficiency of, 253-254

in expression vectors, 323

inducible, 323

in prokaryotes and eukaryotes, 271

recognition sequence, 271

TATA box, 271

tissue-specific, 323

transcription factors and, 271

in viruses, 254, 255 Pronuclei, 339

Proofreading DNA repair, 212 Pro-opiomelanocortin, 720 Prophage, 241, 242 Prophase

meiosis, 168, 170-171

mitosis, 161, 262, 163 Propithecus oerreauxi, 595 Prosimians, 595 Prosome, 555 Prosopis, 687

Prostaglandins, 709, 719, 741, 891 Prostate cancer, 741 Prostate gland, 739, 741 Prosthetic groups, 107, 108 Proteaceae, 1010 Proteads, 1010, 1011 Protease inhibitors

in HIV therapy, 374

in plant defenses, 683, 684 Proteases, 233, 896 Proteasomes, 277 Proteinaceous infective particles, 334 Protein-coding genes

RNA processing, 268-270

structure of, 265-268 Protein kinase cascades, 286-287, 290, 647 Protein kinase receptors, 283-284, 286 Protein kinases, 233, 281

PKA, 290, 291

PKC, 288, 289 Protein phosphatases, 290 Proteins

amino acids in, 36-37

in animal nutrition, 890-891

breakdown during starvation, 888

catabolic interconversion, 131

chaperonins, 42

denaturation, 42

domains, 266

effects of signal transduction on, 291

energy yields from, 887

evolution in, 445-446

functional diversification in, 445

general description of, 36

genetic diseases of, 332-336

interactions with other macro-molecules, 53

noncovalent interactions, 41

peptide linkages, 37-38

polymorphisms, 332

posttranslational modifications, 233,277

posttranslational targeting, 231-233

prions and, 334

prosthetic groups, 36

structure of, 38-40, 41

subunits, 40

surface shape, 40-42

See also Polypeptides Protein starvation, 892, 893 Protein structure

as an evolutionary trait, 431

primary, 38, 39

quaternary, 39, 40, 41

secondary, 38-39

tertiary, 39-40 Protein synthesis

central dogma, 220-221

endoplasmic reticulum and, 64-65

genetic code and, 223-225

Golgi apparatus and, 65, 66

one gene, one polypeptide hypothesis, 218-220

point mutations and, 234-235

posttranslational events, 231-233

regulating in prokaryotes, 249-254

ribosomes and, 64

transcription, 222-223

translation, 225-231 Proteobacteria, 469 Proteoglycans, 77 Proteolysis, 233 Prothoracic gland, 716 Prothrombin, 881 Protists, 5,9,10

alternation of generations, 489-490

Alveolata, 484⁸⁷

Chlorophyta, 492-494

Choanoflagellida, 494

contractile vacuole, 72

defined, 476

distinct from fungi, 529-530

diversity of, 483

endosymbiosis and, 494-495

Euglenozoa, 483

examples of, 477

general biology of, 480-482

mutualisms, 983

recurrent body forms, 495—498

Rhodophyta, 491

size range, 240

Stramenopila, 487-491 Protobionts, 452-454 Protozoel, 555 Protoderm, 612, 613 Protonema, 506 Protonephridia, 914 Proton-motive force, 126-127,

146 Proton pumps

in active transport by plants, 622

in plant guard cells, 627, 628

respiratory chain, 126, 127, 128 Protons, 17, 18, 19

respiratory chain, 125, 126-129 Protoplasts, 316 Protopteris, 432

Protostomes

developmental characteristics,

545 evolutionary trends, 574-575,

577 general characteristics of, 574 major lineages, 553 shared, derived traits, 552 subgroups and number of living species, 578

Prototheria, 593

Prototrophs, 218

Protozoans, 480

Provirus, 243, 244

Proximal convoluted tubule, 919, 920

Prozac, 792

PrP c ' 334

PR proteins, 680, 681

PrP sc ' 334

Prusiner, Stanley, 334

Przewalski's horse, 2009

Pseudacris triseriata, 980, 981

Pseudocoel, 544, 545

Pseudocoelomates, 544, 545

Pseudogenes, 267, 268, 441, 446

Pseudohermaphrodites, 726

Pseudomonas

nitrogen metabolism and, 455 P. aeruginosa, 58, 157, 239

Pseudomyrmex, 984

Pseudopeptidoglycan, 463

Pseudoplasmodium, 497-498

Pseudopodia, 73

Pseudopods, 480, 495, 496, 833

Pseudostratified epithelium, 695, 696

Pseudotsuga menziesii, 620

Psilotophyta, 502, 513
 Psilotum, 512-513
 Pterobranchs (Pterobranchia), 555, 556, 574, 578
 Pterois volitans, 981
 Pterophyta, 502, 510, 513-515
 PTI. See Phosphatidylinositol
 Puberty, sex steroids and, 726-727
 Pubis, 843
 Public policy, biology and, 14
 Puffer fish, 847, 907
 Pulhdaria, 988
 Pulmonary arteriole, 859
 Pulmonary artery, 871
 Pulmonary capillaries, 857, 859
 Pulmonary circuit, 868
 evolutionary trends, 869-871
 Pulmonary valves, 871, 872
 Pulmonary veins, 871
 Pulmonary venule, 859
 Pulp cavity, 894, 895
 Pulse, 872
 Punnett, Reginald C, 180, 190, 400
 Punnett squares, 180-181
 Pupa, 572, 717
 Pupil, 808
 Purines, 47, 131, 203, 204, 205, 790
 Purkinje fibers, 874
 Purple bacteria, 469
 Purple nonsulfur bacteria, 464 165
 Pus
 Pj cnogonida (Pj cnogonida Pygosce/ts papi ">"-Pyloric sphincter, 898 899 Pj ramids of biomass, 997 Pyramids of energy
 Pyrenestes ostrinus, 406, 407 Pj ridoxine, 892 Pyrrolidines, 47, 131,203,204,
 Pj togens
 Pj rophosphatase, 214
 Pj rrophj ta, 477
 Pyruvate, 104,115 in fermentation, 129, 130 in glycolysis, 117, 118,120,121
 Pyruvate dehydrogenase complex, 122
 Pyruvate kinase, 121
 Pyruvate oxidation, 117, 118, 122, 123, 134

Pythons, 589, 704

Qio, 700

Quaking aspens, 960 Qualitative analysis, 28 Quantitative analysis, 28 Quantitative characters, 189 Quaternary period, 380-381, 383,

390 Quaternary protein structure, 39,

40,41 Queen Anne's lace, 522 Quinones, 683

Rabbits

cecum in, 903

digestive system, 895

eye-blink reflex, 827

inheritance of coat color, 186 Rabies, 364

Radial cleavage, 545, 757 Radial symmetry, 546 Radiation

mutation effects, 236

in thermoregulation, 702, 703 Radicle, 650

Radioactive isotopes, 380-381 Radioisotopes, 19, 20 Radiolarians, 481, 496 Radius, 843 Radula, 560, 895 Rainbow trout, 112

Rainfall. See Precipitation Rainforests, 413 Rain shadows, 992 Rana pipiens, 167 Random genetic drift, 402-404,

439 Range, influence on speciation,

421 Rangifer taradmts, 594, 967 Rapid-eye movement (REM)

sleep, 825, 826 Raspberries, 525 Ras protein, 286 Rats

competitive interaction, 977-978

hormonal control of sexual behavior, 931-932 Rat snakes, 894 Rattlesnake bites, 364 Rattlesnakes, 812 Ray-finned fishes, 577

diversity in, 586

swim bladders, 585-586 Ray flowers, 522 Rays, 410, 584, 585

adaptations for water conservation and salt excretion, 917

cartilaginous skeleton, 843

fertilization in, 737 Rb gene, 345 Reactants, 25 Reaction center, 142 Reactions, chemical, 19-20 Realized niches, 975, 976 Receptacle, 521, 522, 666 Receptive fields

of retinal ganglion cells, 810-812

of visual cortex, 823 Receptor cells, 773 Receptor-mediated endocytosis,

91,92 Receptor potential, 796 Receptor proteins, 280

binding of ligands to, 282

in E. coli, 281

genetic diseases of, 333-334

in sensory transduction, 795, 796

types and locations of, 282, 283-285 Recessive traits

diybrid crosses, 182, 183

pedigree analysis, 185 Reciprocal altruism, 955-956 Reciprocal crosses, 177 Reciprocal translocations, 236 Recognition sequences, 260, 271 Recognition site, 313 Recombinant DNA

creating, 313-314

in gene therapy, 347

inserting into cells, 316-317

See also DNA technology Recombinant frequencies, 192 Recombinant phenotypes, 182 Recombination, 191-192

during bacterial conjugation, 246, 247

homologous, 320-321

in immunoglobulin genes, 369

in paramecia, 487 Rectal salt glands, 917 Rectum, 895, 897 Red abalone, 948-949 Red algae, 477, 491, 495, 496 Red blood cells, 259, 355

functions of, 879

glucose-6-phosphate dehydrogenase mutation and, 234

osmosis, 86

production of, 880, 881

sickle-cell anemia and, 234, 235

transport of carbon dioxide, 862, 863

transport of oxygen, 860-862 Red kangaroo, 594 Red light. See Phytochromes Red mangroves, 7 Red muscle, 840 Redox reactions, overview of,

115-116 Red plant kingdom, 501 Red tide, 484 Reducing agents Reducing atmosphere, 451 Reduction, chromosome, 115 Redundancy, in genetic code, 224 Red-winged blackbirds, 414, 952 Reefs. See Coral reefs Reflexes

autonomic, 903

conditioned, 827

diving reflex, 884

Hering-Breuer reflex, 864

knee-jerk reflex, 817, 845

spinal, 817, 919 Regeneration, in animal asexual

reproduction, 733 Regulated growth, 5-6. See also

Development Regulative development, 759 Regulators, 271-272 Regulatory genes, 252 Regulatory systems

properties of, 698-699

vertebrate thermostat, 707-710 Reindeer "moss," 540 Reissner's membrane, 803, 804 Release factor, 229 Releasers, 927-928, 929 Religion, scientific creationism

and, 13-14 REM sleep, 825, 826 Renal artery, 919 Renal corpuscle, 916 Renal pyramids, 919 Renal tubules, 915, 916 Renal vein, 919 Renin, 922

Repetitive sequences, 263-265 Replication complex, 209, 210 Replication forks, 209, 210, 211,

212 Replicator molecules, origin of

life and, 452, 453⁵⁴ Replicons, 315 Reporter genes, 317 Repressible enzyme synthesis,

252-253 Repressors, 251-253, 254, 272 Reproduction

trade-offs with growth, 968-969

See also Asexual reproduction; Plant reproduction; Sexual reproduction Reproductive isolating mechanisms, 418-420 Reproductive signals

cell division and, 155

in prokaryotes, 156

Reproductive system, 698 female sex organs and function, 741-742 hormonal control of male

function, 741 male sex organs and function,

739-741 ovarian and uterine cycles, 742-745 Reproductive technologies artificial insemination, 748 gamete intrafallopian transfer,

749 genetic diseases and, 749-750 intracytoplasmic sperm injections, 749 in vitro fertilization, 748-749 Reproductive value, 969-970 Reptiles (Reptilia), 436, 578 characteristics of, 589 circulatory system, 869-870 development of shelled egg,

737-738 gastrulation in, 763-764 heat production in, 704 major lineages, 589-591 origins of, 588

as a paraphyletic group, 589 Rescue effects, 965 Reserves

economic lands and, 1042 priorities for locating, 1040, 1042 Residual volume, 857 Residues, 36 Resistance vessels, 876 Resolution, 56 Resorption, 911, 920 Resources

in ecology, 975 limiting, 975-976 Respiratory chain

chemiosmotic mechanism,

127-129 electron transport, 125-126,

127 overview of, 117, 118, 125 proton-motive force, 126-127 Respiratory distress syndrome,

858 Respiratory gas exchange adaptations for, 852-857 blood transport of respiratory

gases, 860-863 mammalian lungs, 856-859 physical properties of gas

exchange, 849-852 ventilation and perfusion in, 853 Respiratory organs

adaptations for gas exchange,

852-853 bird lungs, 854-856 fish gills, 853-854, 855 insect tracheae, 853 mammalian lungs, 856-859 tidal breathing, 856-857 Respiratory system

anatomy of, 857, 858-859 blood transport of respiratory gases, 860-863

breathing cycle, 863-864

cilia in, 83L 832

innate defenses, 356

surfactants and mucus in, 857-858

tidal breathing, 856-857, 858-859 Resting potential

ion channels and, 778

measuring, 777

Nernst equation, 779 Restoration ecology, 1042-1043 Restriction endonucleases, 312,

313, 314 Restriction fragment length polymorphisms (RFLPs), 337 Restriction site, 313 Reticular activating system, 818 Reticular connective tissue, 696 Reticular system, 818 Reticulum, 902 Retina, 806

cellular structure, 808-809

information flow in, 809-810

information processing in, 810-812 Retinal, 474, 805, 809 Retinal ganglion cells, 810 Retinoblastoma, 345 Retinol, 892 Retinula cells, 807 Retrotransposons, 265 Retroviruses

HIV, 373-374

reproductive cycle, 243, 244

RNA replication and protein synthesis in, 221 Reverse transcriptase, 321 Reverse transcriptase inhibitors,

374 Reverse transcription, 374 Reversible reactions, 29 Rev protein, 374 R factors, 248-249 RFLPs. See Restriction fragment

length polymorphisms R genes, 681 R groups, 36-37, 105 Rhabdom, 807 *RJwgoletis pomonella*, 418 Rheas, 447, 448

Rhesus monkeys, 267, 431, 908 Rheumatoid arthritis, 359, 372 Rhinocodon typhus, 585 Rhinoceroses, 1038 Rhizobium, 469, 640, 641, 642, 983 Rhizoids, 504, 531 Rhizomes, 508-509, 510, 676 *Rhizopus*, 534, 535

R. oligosporus, 166 Rhodinus, 715-717 Rhodophyta, 477, 491, 501 Rhodopsin, 805-807, 809 Rhynchocoel, 557 Rhynia, 509

Rhyniophytes (Rhyniophyta), 508⁵⁰⁹

whisk ferns and, 512-513 Rhythm method, 745 Ribbon worms, 557-558, 578 Riboflavin, 892 Ribonucleic acid. See RNA

Ribonucleoside triphosphates,

215, 222 Ribonucleotides, 48 Ribose, 43, 44, 47, 220 Ribosomal RNA (rRNA), 64, 222

in evolutionary molecular biology, 447, 467

gene amplification, 275

moderately repetitive DNA sequences, 264

transcription of, 222 Ribosomes

component rRNA molecules, 264

endoplasmic reticulum and, 64

in plant cells, 61

polysomes, 230-231

in prokaryotes, 58

structure and function, 64, 226-227

in translation, 225, 227-229 Ribozymes, 102, 229, 322, 454 Ribs, 843

Ribulose biphosphate carboxylase/oxygenase (Rubisco)

Calvin-Benson cycle and, 147

in C 4 plants, 150

in photorespiration, 148-149 Ribulose 1,5-bisphosphate

(RuBP), 147, 148-149 Ribulose monophosphate

(RuMP), 147, 148 Rice

chromosome pairs, 167

foolish seedling disease, 651 Rickets, 892

Rickettsia proivazekii, 256, 446 Riftia, 559 Right whales, 887 Rigor mortis, 838 Ringed kingfisher, 996 Risk costs, 948 Rivers, cycling of materials,

999-1000 RNA polymerases, 261, 265

in eukaryotes, 271

in transcription, 222, 223 RNA primase, 209, 211, 222 RNA primer strands, 210-211, 222 RNA (ribonucleic acid)

antisense, 322

base pairing, 48

catalyst activity during translation, 229

central dogma and, 220-221

compared to DNA, 47, 220

nitrogenous bases in, 47, 48

nucleic acid hybridization, 268

nucleotide components, 47-48

origin of life and, 453-454

primary transcript processing, 268-270

reverse transcription, 243, 244

ribose in, 43, 47

viroids, 244 RNA viruses, 221, 241 Roaches, 572

Rocks, weathering of, 638 Rod cells, 809, 820

anatomy of, 805

response to light, 806-807

Rodents, 595

competitive interaction, 977-978 Rodhocetus, 385 Romalea microptera, 842 Romanov family, 328-329 Root cap, 622, 613

Root hairs, 614, 642 Root nodules, 640, 641, 642 Root pressure, 624-625 Roots/Root systems, 604, 605

apical meristems, 612, 613

Casparian strips, 623

in dry environments, 686-687

embryonic, 650

endodermis, 623

evolution of, 510

ion exchange and, 639

mycorrhizae, 532, 539-540, 614, 641, 983

nitrogen-fixing nodules, 640, 641, 642

secondary growth, 615-616

suckering, 676

tissues in, 613-614

uptake and transport of water and minerals, 620-624

in water-saturated soils, 687, 688

zones of, 613 Roquefort cheese, 536 Rosa, 434

R. rugosa, 527 Rosaceae, 434, 527 Rosales, 434 Roses, 527

Rosy periwinkle, 2032 Rotational cleavage, 758 Rotifers (Rotifera), 554-555, 574,

578 Rough endoplasmic reticulum,

60, 61, 64, 65, 66 Round dance, 936 Roundup, 327 Round window, 804 Roundworms, 566-567, 578. See

also Nematodes Rous, Peyton, 343 rRNA. See Ribosomal RNA RT-PCR, 321 RU-486, 746, 747 Rubisco. See Ribulose

bisphosphate carboxylase/oxygenase RuBP. See Ribulose 1,5-bisphosphate Ruffini's corpuscle, 800 Rumen, 466, 902

Ruminants, 466, 902-903 Runners, 605, 606 Rye, 675

Saccharomyces, 315, 532 Saccharomyces cerevisiae, 535, 536

DNA rearrangements in, 275

genome, 262, 446

mating types, 275 Saccoglossus kowaleskii, 582 Sacrum, 843 Saddle joint, 845 Saguaro cacti, 7, 959, 961

Saint-Hilaire, E. Geoffroy. See

Geoffroy Saint-Hilaire, E.

Sake, 536

Sakmann, Bert, 783

Salamanders, 411, 587, 737, 980

Salamandra salamandra, 588

Salicylic acid, 647, 660, 680, 681

Saline environments, plants in, 688-689

Saliva, 356

Salivary amylase, 111, 902

Salivary glands, 715, 897, 901

Salivation, 903

Salix, 680

Salmon, 586, 714, 737

Salmonella typhimurium, 460

Salsifies, 417

Saltatory conduction, 784-785

Salt bridges, 40

Salt concentrations, 911

Salt glands, 688 nasal, 912, 923 rectal, 917

Salviniales, 514

Samaras, 670

Sanctuaries, priorities for locating, 1040, 1042

Sand dollars, 581

Sandpipers, 22

San Joaquin kit fox, 707

Saprobies, 490, 529, 531-532, 893

Saprolegiia, 490

Saprotrophs. See Saprobies

Sapwood, 617

Sarcomas, 343

Sarcomeres, 836, 837

Sarcophilus harrisi, 594

Sarcoplasm, 838, 839

Sarcoplasmic reticulum, 836, 838, 839, 840

Sarcoscypha coccinea, 536

Sargassum, 489

Sarracenia, 644

Satellites, 263

Saturated fatty acids, 50

Savannas, 1023

Sawflies, 574

Scale-eating fish, 409

Scanning electron microscopy, 57

Scaphiophryne gottlebei, 588
 Scapula, 843
 Scarab beetles, 704
 Scarlet gilia, 682
 Schally, Andrew, 721
 Schistosoma, 425, 426
 Schistosomiasis, 425
 Scholander, Per, 620, 626
 Schools, fish, 586
 Schwann cells, 776, 784
 Scientific creationism, 13-14
 Scientific inquiry
 creation science and, 13-14 experimentation, 12-13 hypotheses and the hypotheti-co-deductive method, 10-12
 Scientific names, 10
 Scion, 677
 Sclereids, 608, 609
 Sclerenchyma, 608, 609, 610
 Scleria, 406
 Sclerotium, 497
 tpendra hems, 571 Scorpions 570 Scots pine
 tiring rushes, 512 pie, 334 Scrotum, 739, 740 Scui 992
 tozoans (Scyphozoa), 550, >78 Sm anemones, 54S, 551 hydrostatic skeleton, 841 nen e net, 774
 Sj mbiosis w ith green algae, 69 Sea butterflies, 562 Sea cucumbers, 580, 581 Sen lev els
 changes in Earth's history, 383 global warming and, 1003 Sea lilies, 580-581 Seals, diving reflex, 866, 884 Sea nettle jellyfish, 548 Sea otters, 1039 Sea palms, 489 Sea slugs, 563 Sea snakes, 2027 Seasonal changes, photoperiodic-
 itv and, 727 Sea squirts, 430, 431, 582-583 Sea stars, 579, 580, 581, 733, 894,
 986-987 Sea turtles, 590 Sea urchins, 580, 581
 blocks to polyspermy, 754, 755 cleavage in, 757 early embryo asymmetry, 301 fertilization in, 753 gastrulation in, 759-761
 Secondary active transport,
 89-90 Secondary carnivores, 995, 996 Secondary compounds, 682-683, 982 plant protection from, 684-685 Secondary
 growth, 518, 613,
 615-617 Secondary immune response,
 360 Secondary lysosomes, 67 Secondary oocytes, 735, 736, 742 Secondary phloem, 615, 626, 617 Secondary plant body, 612
 Secondary protein structure,
 38-39 Secondary sex determination,
 194 Secondary spermatocytes,
 735-736, 740 Secondary succession, 987, 988 Secondary walls, 607 Secondary xvlem, 518, 615, 616,
 617 Second filial generation (F2), 179 Second messengers calcium ions, 288-289 cAMP, 287 from lipids, 287-288
 metabotropic receptors and,
 788, 789 nitric oxide, 289 Second polar body, 735, 736 Secretin, 719, 903 Secretion, 911
 exocytosis, 91-92

Seed banks, 1022 Seed cones, 518, 520 Seedlings

apical hook, 659

etiolated, 662

food reserves and early growth, 650, 652

gravitropism in, 654

phototropism in, 653, 654 Seed plants

life cycle, 516-518

phyla of, 502, 516, 527

See also Flowering plants; Gymnosperms Seeds

anatomy of, 670

asexual production of, 676

of conifers, 519-521

dispersal, 670

dormancy, 647, 649-650, 652, 670

embryo development in, 668-670

germination, 647-648, 649-650

of seed plants, 517-518

sensitivity to light, 661 Segmentation, maternal effect

genes and, 305-306 Segmentation genes, 306 Segmented worms, 578, 732, 733 Segment polarity genes, 306 Sei whales, 972 Seizures, 827 Selective herbicides, 656 Selenium, 890 Self-compatibility, 432 Self-fertilization, 404, 667 Self-incompatibility, 432, 668 Selfish behavior, 953 Semen, 740-741

Semicircular canals, 801, 802, 803 Semiconservative replication

defined, 206

mechanisms of, 208, 209-212

Meselson-Stahl experiment, 206, 207-208 Seminal vesicles, 739, 740 Seminiferous tubules, 739-740 Sempewivum tectorum, 151 Senescence, 648-649, 970 Senescence hormone. See

Ethylene Sensory cells, 773

adaptation, 797

properties of, 794

response to stimuli, 794, 795

in sensory circuits, 795

in sensory organs, 795

sensory transduction, 795-796 Sensory circuits, 795 Sensory organs, 795 Sensory systems

adaptation, 797

auditory, 803-804

chemoreceptors, 797-799

detection of electrical fields, 812

detection of ultraviolet and infrared light, 812

echolocation, 812

mechanoreceptors, 799-804

sensory cells, 794-795

sensory circuits, 795

sensory organs, 795

sensory transduction, 795-796

visual, 805-812 Sensory transduction, 795-796 Sepals, 522, 666, 672 *Sepia latimamis*, 847 Septa, 531 Septate hyphae, 531 Sequence-tagged sites, 349 Sequential hermaphrodites, 736 *Sequoiadendron giganteum*, 519 *Sequoia sempervirens*, 620 Sergeant major fish, 956 Serine, 37, 224 Serine kinase receptors, 283 Serosa, 897 Serotonin, 789, 792 Sertoli cells, 739-740 Sesquiterpenes, 683 Sessile animals, 574-575 Setae, 558 Set points, 698, 699

vertebrate thermostat and, 708, 709 Sex cells, 5 Sex chromosomes, 165

functions of, 194

inheritance of genes, 195-196

nondisjunctions, 194

sex determination, 194 Sex determination, 192, 194 sex-lethal (sxl) gene, 934 Sex-linked inheritance, 195-196 Sex organs

accessory, 737

development of, 726

female, 741-742

male, 739-741 Sex steroids, 725

effects of, 726

male sexual function and, 741

ovarian and uterine cycles and, 743, 744-745

in puberty, 726-727

sexual behavior and, 931-932 Sexual behavior

hormonal control of, 931-932

human, 745-750

parthenogenetic reproduction and, 734

sexually transmitted diseases and, 750 Sexual development

puberty, 726-727

sex organs, 726 Sexual excitement, 745 Sexually transmitted diseases

(STDs), 748-749, 750 Sexual reproduction

alternation of generations, 167

in diatoms, 488

diplontic life cycle, 167

emergence of, 4

in fungi, 533

genetic diversity, 165

genetic variation and, 408

hallmarks of, 165

haplontic life cycle, 167

in plants, 666-670

in protists, 482 Sexual reproduction (animal)

gametogenesis, 734-736

genetic diversity and, 734

hermaphroditism, 736 human reproductive system,

739-745 mating systems, 736-737, 738 overview of, 734 oviparity, 738-739 shelled egg, 737-738 viviparity, 739 Sexual responses, 745 Sexual selection, 950-951 S gene, 668 Sharks, 584-585

adaptations for water conservation and salt excretion, 917 cartilaginous skeleton, 843 fertilization in, 737 intestines of, 896 Sheep, 334 Shelled egg

development of, 737-738 oviparity, 738-739 Shigella, 249 Shivering, 706, 708 Shoots/Shoot systems, 604, 605-606 apical meristem, 612, 614 development patterns, 648 rooting with auxin, 655 Short-day plants, 671, 672-673 Short interspersed elements

(SINEs), 264-265 Short-long-day plants, 672 Short-term memory, 819, 828 Shotgun DNA sequencing,

348-349 Shrews, 595 Shull, G. H., 188 Siamese cats, 189 Siberian hamster, 728 Sick-cell anemia, 234, 235, 332-333 comparison with phenylketonuria, 338 DNA testing for, 341, 342 identifying mutant gene in, 336 Side chains, 36-37 on enzymes, 105 hydrophobic, 40 Sieve plates (echinoderms), 579 Sieve plates (phloem), 610 pressure flow model and, 630-631 Sieve tubes, 610

in phloem transport, 629, 630, 631-632 Sieve tube sap, 610 Sifaka lemur, 595 Signal amplification, 796, 807 Signal recognition particle, 233 Signals

in animal communication,

935-937 cellular, 279-282 See also Cell signaling;

Hormones; Signal transduction pathways Signal sequences, 231 Signal transduction pathways direct and indirect transduction, 285 effects of, 290-292

essential components of, 281-282

for ethylene in plants, 659-660

hormones and, 728

in plant development, 647

protein kinase cascades, 286-287

receptors, 282-285

regulation of, 290

signal types and sources, 279-281

transducers, 285-290 Signature sequences, 467 Silencers, 261, 271, 272 Silent mutations, 234 Silent substitutions, 439 Silk protein, 311 Silkworm moths, 716, 717, 797 Silt, 638

Silurian period, 380-381, 387, 508 Silverfish, 573 Silverswords, 422, 423 Silver thiosulfate, 659 Similarity matrices, 440, 441 Simple cells (visual cortex), 823 Simple diffusion, 86, 89 Simple epithelium, 695, 696 Simple fruits, 525 Simple leaves, 510, 605-606 Simple lipids. See Triglycerides Simple tissues (plant), 610 Simultaneous hermaphrodites,

736 SINEs, 264-265 Sinks, in plants, 629 Sinoatrial node, 873, 874 Siphonaptera, 574 Siphons, 562 Skates, 410, 584, 585 Skeletal muscles, 697, 834

contraction in, 835-839

fast-twitch and slow-twitch fibers, 840-841

flexor and extensor, 817-818, 845

joints and, 845-846

shivering, 706

stretch receptors and, 800, 801

summing of muscle twitches, 839-840 Skeletons/Skeletal systems

cartilage and bone in, 696, 697

hydrostatic, 841-842

organs in and functions of, 698

types of, 546

See also Endoskeletons; Exoskeletons Skin, 698

innate defenses and, 356

tactile mechanoreceptors in, 799-800

in thermoregulation, 702-703, 704, 707, 708-709 Skin pigmentation, 189

evolution of, 891-892 Skull, 843 Skull cap, 844 Skunks, 847 Sleeping, 825-826 Sleeping sickness, 483, 484 Sleepwalking, 825

Sliding filament theory, 837-839 Slime molds, 495, 496-498

distinct from fungi, 529-530 Slow block to polyspermy, 754,

755 Slow-twitch muscle fibers, 840,

841 Slow-wave sleep, 835 Slug (pseudoplasmodium),

497-498 Slugs, 562, 562 Small intestines, 897

cholecystikinin and, 903 digestion in, 899-900 digestive enzymes from, 901 hormones of, 719 innate defenses in, 356
nutrient absorption in, 900-901 Small nuclear ribonucleoprotein

particles (snRNPs), 269 Smallpox, 353, 360 Smell. See Olfaction Smith, Hamilton, 255 Smooth endoplasmic reticulum,

60, 61, 65 Smooth muscles, 697

in arteries and arterioles, 875,

876 in autoregulation of the circulatory system, 882 contraction, 289, 834, 835, 839 gut and stomach, 697-698, 897 in veins,
878 Snails, 562

cleavage and, 758 evolution in, 393 schistosomiasis and, 425 Snakes, 589

feeding adaptations, 893-894 fertilization in, 738 heat detectors, 812 olfaction in, 799 Snapdragons, 186, 187 Sneezing, 356
Snowshoe hares, 979, 980 snRNPs. See Small nuclear

ribonucleoprotein particles Social behavior

altruism, 953, 954-956 costs and benefits of, 952-953 types of, 953-954 Sodium, 24

absorption in small intestine,

901 aldosterone and, 726 in animal nutrition, 890 membrane potential and, 777 NMDA receptors and, 790, 791 Sodium
channels

in action potentials, 780, 781,

782, 783 in depolarization and hyper-polarization, 780 in phototransduction, 806, 807 in sensory transduction, 795, 796
Sodium cotransport, 901 Sodium-potassium pumps, 89, 90, 778

Soft palate, 898 Soft-shelled crabs, 843 sog gene, 543 Sog protein, 309 Soil communities, atmospheric carbon dioxide and,
1003 Soil erosion, mercury pollution

and,991 Soil nitrogen, organic matter

and,639 Soil organic matter, 638, 639 Soil pH

effect of plants on, 640

liming and, 639

sulfur and, 639 Soil profile, 638 Soils, 1001

composition and structure, 637-638

effect of plants on, 639-640

formation of, 638

mineral nutrients and ion exchange in, 638-639

of tropical deciduous forests, 1024 Soil solution, 634 Solatium tuberosum, 167 Solar compass, time-compensated, 942 Solar energy, 992 Sole, 410

Solute potential, 621, 911 Solutions, 28 Somatic cells, 734 Somatic mutations, 234 Somatostatin, 728, 722, 724 Somites, 766 Songs

of whales, 936

See also Bird songs Song sparrows, 952, 966, 967 sonic hedgehog gene, 766 Sonoran Desert, 959 Soredia, 541 Sorghum, 252 Sori, 513, 524

Sound, in animal communication, 936 Sources, in plants, 628-629 South Africa, fynbos vegetation,

2022, 1032-1033 South America, biota of, 1010 Southern beeches, 1017 Southern Indian Lake, 991 Southern Sea Otter Recovery

Team, 1039 Sow bug, 570 Soy sauce, 536 Spatial learning, 928-929 Spatial summation, 788, 840 Spawning, 712 Speciation, 6-7

allopatric, 414-417

evolutionary radiation, 422²³

molecular studies in, 419-420

in mosquitos, 413

parapatric, 418

reproductive isolating mechanisms, 418-420

significance of, 423

stasis in, 391 sympatric, 417-418 Speciation rates behavior and, 421 environmental influences, 421 evolutionary radiation, 422-423 generation times and, 421-422 range size and, 421 species richness and, 420 Species

ancestral and derived, 7 binomial nomenclature,

433-434 definitions of, 413¹¹⁴ effects on community structure, 986-987 endemic, 422, 1009-1010 forming of, 6

molecular studies of, 419-420 stasis in, 391 Species-area relationship,

1030-1031 Species distribution, 1008-1011.

See also Biogeography Species extinctions amphibians, 587-588 biodiversity loss and, 1043 estimating current rates of, 1030-1032 global warming and,

1038-1039 habitat destruction, 1034 habitat fragmentation,

1034-1036 human-caused, 393 introduced pests, predators,

and competitors, 1037 overexploitation, 1038 preventing, 1039-1040 proportion of U.S. species extinct or at risk, 2034 significance of, 1032-1033 Species pool, 1012 Species preservation, habitat

and, 971 Species richness, 986

island biogeographic model,

1012-1014 latitudinal variation, 1014 Pacific reefs and, 2027 Species-specific behavior, 926.

See also Inherited behavior Species-specific coevolution,

985-986 Specific defenses, 354. See also

Immune system Specific heat, 27, 993 Spemann, Hans, 762 Spergula vernalis, 963 Sperm

blocks to polyspermy, 754, 755 capacitation, 754 contributions to zygote,

754-755 egg-sperm recognition mechanisms, 753-754 fertilization in humans,

741-742 flagella and, 832 formation of, 735-736, 739-740

maturation, 40 mechanisms of Fertilization

motility of, 4

in semen, 739, 741 Spermatid- 735 736 740 Spermatocytes, 735-736, 740 Spermatogenesis ~ ;% 736,

739-740, 741 Spermatogonia, 735 Spermatophores, 737 Sperm cells (plant), 668, 669 Sperm costs, breeding behavior

and, 950 Spermicides, 746 Sperm whales, 971 Sperry, Roger, 828 Sphagnum, 500, 507 S phase, 158 Spkenodon punctatus, 590 Sphenodontida, 589 Sphenophyta, 501, 510, 512 Spherical symmetry, 546 Sphincter muscles, urinary, 919 Sphinx moths, 704 Sphygmomanometer, 872, 873 Spices, 974, 982 Spicules, 347

Spider monkeys, 431, 1036 Spiders, 569, 570

feeding adaptations, 894

fertilization in, 737

fossilized, 385

web spinning behavior, 926 Spider silk, 34, 311 Spider webs, 407-408, 569, 926 Spike inflorescences, 522 Spina bifida, 766 Spinal cord, 774

anatomy and functioning, 817^818

autonomic nervous system and, 822-823 Spinal nerves, 816, 817 Spinal reflex, 817

urination and, 919 Spindles, mitotic, 161, 262, 163,

164 Spiny anteaters, 593 Spiracles, 853 Spiral cleavage, 545, 758 Spiralian, 555, 557-558 Spirobranchus, 559 Spirochetes, 240, 462, 470-471 Spirometers, 856, 857 Spiteful behavior, 953 Spleen, 881 Spliceosomes, 269 Splicing

alternate, 276

in RNA processing, 269-270 Split-brain persons, 828 Sponges, 494, 545

cell adhesion in, 82-83

gas exchange and, 850

general biology of, 546-547

regeneration in, 733

subgroups and number of living species, 578 Spongy mesophyll, 618 Spontaneous abortion, 747

Spontaneous generation, 455, 456 Spontaneous mutations, 236, 237 Spontaneous reactions, 99 Sporangia

terns, 513, 514

nontracheophytes, 504

plant life cycle, 501

in slime molds, 497 Sporangiphores, 497, 534 Spores, 165, 166

cyanobacteria, 470

fungi, 529

prokaryotes, 5 Sporocytes, 490 Sporophytes

angiosperms, 521, 524

conifers, 520

defined, 490, 501

ferns, 513-515

flowering plants, 666, 667

homospory and heterospory, 511

hornworts, 506

mosses, 506

nontracheophytes, 504
 plants life cycle, 501
 seed plants, 517
 tracheophytes, 507 Sporozoites, 485 Spotted puffer fish, 577 Springtails, 1003 Spring wheat, 675 Squamata, 589 Squamous epithelial cells, 695,
 696 Squids, 562
 fertilization in, 737
 giant axons, 783
 hydrostatic skeleton and propulsion in, 841-842
 nervous system, 774 SRY gene, 194
 Stabilizing selection, 405-406 Staghorn coral, 552, 737 Stahl, Franklin, 207 Stained bright-field microscopy,
 57 Stamens, 521, 666, 671
 evolution of, 522, 523 Stanley, Wendell, 240 Stapes, 802, 803 Staphylococcus, \7\ -472
 S. aureus, 472 Staplnjlothermus marinus, 465 Starches
 biological functions, 45-46
 in plants, 147
 in seeds, 650
 structure, 45
 types of, 46 Starch grains, 70 Star maps, 942-943 Start codon, 223, 224, 229, 230 Starvation, 888-889 Statocysts, 801
 Statoliths, 801 Steele, 614, 622-624 Steller's sea cow, 1038 Stem cells
 gene therapy and, 347
 manipulating differentiation in, 299
 medical uses, 294
 potential for use in medicine, 294, 299, 300
 production of megakaryocytes, 880
 production of red blood cells, 880, 882 Stems, 604, 605
 ethylene and, 659
 primary tissues, 612-613, 614-615
 secondary growth, 615-617
 shoot apical meristem, 612, 614
 underground, 676 Steno, Nicolaus, 380 Stereocilia, 801, 802
 auditory, 803
 microfilaments in, 833
 See also Microvilli Stereotypic behavior, 926. See also
 Inherited behavior Sterilization, 747 Sternum, 843 Steroids, 683
 chemical structure, 51
 kinds of, 52
 receptors for, 284-285
 ring structure, 52 Stethoscopes, 872, 873 Sticklebacks, 391 Sticky ends, of DNA, 314 Stigmas, 522, 522, 666, 668 Sting rays,
 410, 422 Stirrup, 802, 803 Stock, 677 Stolons, 676 Stomachs, 895, 897, 901

digestion in, 898-899
 hormones and, 729, 903
 innate defenses in, 356
 intrinsic factor and, 893
 in ruminants, 902, 903
 tissues in, 695, 697-698 Stomata, 611, 618
 abscisic acid and, 660
 in crassulacean acid metabolism, 628
 in hornworts, 505-506
 transpiration and, 627-628
 in xerophytes, 685-686 Stone cells, 608 Stone flies, 572 Stoneworts, 501, 502 Stop codons, 223, 224, 230 Stotting behavior, 947 Strains, 200 Stramenopiles (Stramenopila), 477, 487-491, 501 Strasburger, Eduard, 624 Stratified epithelium, 695, 696 Stratosphere, 1000 Stratton, Charles, 720 Strawberries, 525 Strawberry blight, 682 Strawberry plants, 605, 606, 625, 666, 676 Streptococcus pneumoniae, 55, 200-201 Streptomyces, 472 Streptomycin, 230, 472 Stress, Cortisol and, 726 Stress response elements, 272, 273 Stretch receptors, 800, 801 Striated muscle cardiac, 834-835 skeletal, 835-839 Strip mining, plants and, 689 Strobili, 512 Strokes, cardiovascular disease and, 879 Stroma, 69 Stromalites, 454, 455 Strongylocentrotus purpuratus, 580 Structural genes, 250 Structural isomers, 32, 43 Structural proteins, genetic diseases of, 334 Sturtevant, Alfred, 192 Styles, 522, 522-523, 666 Suberin, 607, 613 Sublingual salivary gland, 897 Submandibular salivary gland, 897 Submucosa, 897 Subpopulations, 408-409 rescue effects, 965 Subsoil, 638 Substance P, 790 Substitutions. See Nucleotide substitutions Substrate-level phosphorylation in citric acid cycle, 124 in glycolysis, 120 Substrates concentration levels and reaction rate, 105-106 defined, 104 enzyme-substrate interactions, 105 specificity of active site to, 104, 106 Succession, 987-988 Succinate, 223, 124 Succinate dehydrogenase, 109, 124-125 Succinate-Q-reductase, 126 Succinyl CoA, 223, 124, 131 Succulent plants, 151, 686, 689 Suckers, 676 Sucrase, 902 Sucrose, 147-148 translocation in plant phloem, 628-632 Sucrose-proton symport, 631, 632 Sugar bisphosphates, 119 Sugar phosphates, 46 Sugars, translocation in plant phloem, 628-632 Sulci, 819 central sulcus, 820 Sulfate, 639, 643 Sulfhydryl group, 32 Sulfolobus, 473 Sulfur in animal nutrition, 890 metabolism in plants, 643 as nutrient, 634 photosynthetic bacteria and, 464

in plant nutrition, 636

soil pH and, 639 Sulfur bacteria, 454. See also

Cyanobacteria Sulfur cycle, 1004 Sulfur deficiency (plant), 636 Sulfur dioxide, 1004 Sulfuric acid, 1004 Sulfur-oxidizing bacteria, 998 Sumatran pit viper, 590 Sundews, 644 Sunflowers, 422-423 Superficial cleavage, 757 Superior vena cava, 871 Suprachiasmatic nuclei, 937-938,

939 Surface area-to-volume ratio,

55-56 Surface tension, 28 Surfactants, in lungs, 857-858 Survivorship, 962-963 Suspensor, 669 Sutherland, Earl, 287 Swallowing, 897-898 Swarm cells, 497 Sweat glands, 79, 715 Sweating, 79, 707 Swim bladders, 585-586 Sycamores, 418 Symbiotic associations

dinoflagellates in coral, 551

green algae in sea anemones, 69

mycorrhizae, 532, 539-540, 540, 614, 641, 983

in nitrogen fixation, 640, 641 Symmetry, 545, 546 Sympathetic nervous system, 821-823, 873, 882, 883 Sympatric speciation, 417-418

molecular studies, 419-420 Sympetrum uulgatum, 573 Symplast, 623-624, 631, 632 Sympodia, 988 Symport transporters, 89 Synapses ' defined, 785

electrical, 789

excitatory and inhibitory, 786-787

neuromuscular junction, 785-786

overview of, 775-776 Synapsis, 268, 170, 172 Synaptic cleft, 785, 791-792 Synaptonemal complex, 170 Synergids, 666, 667 Syngamy, 493 Synonymous substitutions, 439,

441 Synthetic toxins, 907-908 Syphilis, 748-749, 750 Syrphidae, 573 Systematics

classification systems, 432-434, 435

evolutionary classification, 435-436

future of, 437

phylogenetic trees, 425-430,

431-432 traits used in, 430-431 Systemic acquired resistance,

680-681 Systemic circuit, 868, 869-871 Systemic lupus erythematosus,

372 Systemin, 647, 660, 683, 684 Systems, open and closed, 97,

98-99 Systole, 872, 873

Tachycardia, 874

Tactile communication, 936-937

Tactile mechanoreceptors,

799-800 Tadpoles, 914, 980, 981 Taeniura lymnaea, 411 Tailings. See Heavy metals; Mine

tailings Tannins, 683, 982 Tapeworms, 554, 736 Taproots, 605, 687 Tardigrada, 568, 578 Target cells, 713 Targeting sequences, 323 Tarpon, 996 Tarsal bones, 843 Tarsiers, 595 Tarweed, 422, 423 Tasmanian devil, 594 Taste, 798-799 Taste buds, 798-799 TATA box, 271 Tat protein, 374 Tatum, Edward, 218, 220,

245-246 Tautomers, 236, 237 Taxa. See Taxon/Taxa Taxol, 347, 1043 Taxonomy

defined, 433

evolutionary relationships in, 435-436

Linnean, 433-434, 435

See also Classification systems Taxon/Taxa, 433, 435-436 Tay-Sachs disease, 341 T2 bacteriophage, 201-202 T cells. See Cytotoxic T cells T cell leukemia, 343 T cell receptors, 355, 359, 365-366, 367, 368 T cells, 355

in autoimmunity, 372

cell surface receptors, 365-366

clonal anergy, 361

clonal deletion, 360-361

clonal selection, 359

daughter cells, 360

interactions with antigen-presenting cells, 366, 367

overview of, 354, 359, 365

specificity of, 358

types of, 366 T DNA, 316 Tears, 356

Tectorial membrane, 803 Teeth, 894, 895 Tegetricula, 985-986

Telencephalon, 816 Telomerase, 263, 264 Telomeres, 263-264 Telomeric sequences, 260 Telophase
meiosis, 269, 171

mitosis, 263, 164 Temperate deciduous forests,
1018 Temperate grasslands, 1019 Temperate viruses, 242 Temperature
annual patterns, 1014

circadian rhythms and, 674

effect on enzymes, 112

effects on gas exchange for water breathers, 850-851

life and, 699-700

of ocean waters, 1026

plant adaptations to, 689-690

vernalization in plants, 675-676 Temperature regulation. See
Thermoregulation Temperature sensitivity, 700 Temperature tolerance, fungi
and, 531 Template strand, 222 Temporal lobe, 819, 828, 829 Temporal summation, 788, 840 Tendons, 845

Golgi tendon organs and, 800, 801 Tension, in xylem water transport, 625-626 Tent caterpillars, 978-979 Tepals, 522

Teratocarcinomas, 299 Terminal transferase, 369 Terminator sequences, 230, 265 Termites, 572, 954-955, 983 Terpenes,
683

Terrestrial biomes, 1014-1025 Territory marking, 935 Tertiary period, 380-382, 384, 390 Tertiary protein structure, 39-
40 Test crosses, 181-182 Testes, 724, 734

castration, 741

hormones of, 729

spermatogenesis in, 739-740 Testosterone, 52, 726

brain function in birds and, 932

male sexual function and, 741

rat sexual behavior and, 932, 932

structure of, 725 Tetanus (disease), 467 Tetanus (muscle contraction), 840 Tetra, 996 Tetracycline, 230 Tetrads,
chromosomal, 171 Tetrapods, 587-588 Tetrodotoxin, 847 Texas rat snake, 894 Thalamus, 816, 826 Thalli, 488

of lichens, 541 T H cells. See Helper T cells Theria, 593-594

Thermoclines, 999 Thermodynamics, laws of, 97-99,

994 Thermogenin, 129, 706 Thermoneutral zone, 705-706 Thermophiles, 469, 473 Thermoplasma, 474 Thermoregulation
 behavioral, 702
 classification of animals by, 701
 in ectotherms, 704-705
 ectotherms and endotherms compared, 701-702
 in endotherms, 705-707
 fevers, 709
 heat exchange in, 702-703, 704
 regulated hypothermia, 709-710
 thyroid regulation in, 723
 vertebrate thermostat, 707-710 Thermosensors, 795 Thermostats, properties of, 699 Thermus aquaticus, 216 Thiamin, 892
 Thiocystis, 465 Thiogalactoside transacetylase,
 250 Thiols, 32
 Thiotnargarita namibienses, 459 Thompson, Richard, 827 Thomson's gazelle, 947 Thoracic cavity, 858 Thoracic duct, 877,
 901 Thorax, of crustaceans, 571 Thorn forests, 1023 3' ends, of DNA, 204, 205, 208 Three-spined stickleback, 391
 Threonine, 37, 224, 889, 890 Threonine deaminase, 209 Threonine kinase receptors, 283 Thrips, 572 Thrombin, 95, 881
 Thrombus, 879 Thrum flowers, 404 Thylakoids, 68-69, 146, 470 Thymine, 47, 48, 220
 in DNA, 203, 204, 205
 DNA point mutations and, 338 Thymosins, 728 Thymus, 724 Thyroid gland, 724
 calcitonin and, 723, 724
 goiters, 722-723
 hormones of, 728
 iodine and, 893
 regulation in response to cold, 723
 thyroxine and, 722 Thyrotropin, 728, 719, 721, 722 Thyrotropin-releasing hormone
 (TRH), 721, 722 Thyroxine, 714
 half-life, 729-730
 iodine and, 893
 structure of, 722
 targets and actions of, 728, 722, 723 Thysanoptera, 572 Tianfangia, 387
 lib...
 570 734
 Tidal brtuthn
 control and regulation of,
 Ddal volume, 85 857 ngers, 935
 Tight junctions, 84-85 lime-compensated solar compass, 942 tun gene, 939 Tim protein, 939 Tinbergen, Niko, 927-928 Ti
 plasmid, 316 Tip layers, 676 Tissue culture. See Plant tissue
 culture Tissue fluids, 867
 lymphatic system and, 877
 plasma and, 882
 regulation of composition, 912

water balance and, 910-911 Tissue plasminogen activator,
 323, 324 Tissues (animal), 8
 connective, 696-697
 diffusion within, 85
 epithelial, 695
 muscle, 697
 nervous, 697
 in organs, 695, 697-698
 types, 695 Tissue-specific promoters, 323 Tissues/Tissue systems (plant), 610
 primary meristems and, 613
 roots, 613-614
 stems, 614-615 Tmesipteris, 512
 TMV. See Tobacco mosaic virus Toads, 167, 411, 587, 914 Tobacco mosaic virus (TMV),
 240, 632, 681 Tobacco plants, 632, 671, 672 Tocopherol, 892 Tomatoes, 690 Tongue, taste buds, 798-799 Tonicella lineata,
 561 Tonoplast, 610 Tonus, 840 Topsoil, 638 Torpedo, 847 Tortoises, 589-590 Total lung capacity, 856 Totipotent 296, 297-
 299 Townsend's warblers, 419, 420 Toxins
 acute, 982
 bacterial, 326, 467
 bioaccumulation of, 907
 biological detoxification, 907
 rnimicry of hormones, 907-908
 from poison glands, 847
 retained and concentrated in organisms, 906-907 T7 phage, 312 Trachea, 572, 852, 898
 in birds, 854
 of insects, 853
 in mammals, 857, 858 Tracheary elements, 608
 [racheids, 507, 518, 608, 609 Tracheoles, 853 Tracheophytes
 defined, 501
 early forms of, 508-509
 evolution of, 508
 nonseed members, 501, 507-515
 phyla of, 501
 vascular system in, 502-503 Tragopgon, 417 Trail marking, 935 Traits
 ancestral and derived, 427, 428
 constructing a simple phy-logeny with, 428-430
 defined, 428
 determining evolution of, 432
 in genetics, 178
 homologous, 427

homoplastic, 427 Transcription, 220, 222-223
eukaryotic, 262, 266
initiation, 271, 274
point mutations and, 234-235
start and stop signals in, 230 Transcriptional regulation
chromatin structure and, 273-275
contrasting in eukaryotes and prokaryotes, 270-271
coordinating several genes, 272-273
DNA-binding proteins, 273
energy conservation with, 250
gene amplification, 275-276
negative, 254
operator-repressor systems, 251-253
positive, 254
promoter systems, 253-254
regulators, enhancer, and silencers, 271-272
signal transduction and, 291-292
structural genes and promoters, 250-251
transcription factors, 271
in viruses, 254, 255 Transcription errors, 223 Transcription factors, 271, 300,
308 Transducers, 285 Transducin, 806, 807 Transduction, 247 Transfer cells, 623-624 Transfer factor, 228 Transferrin, 881
Transfer RNA (tRNA)
activating enzymes, 226, 227
anticodons and amino acid specificity, 225-226, 227
in the central dogma, 221
moderately repetitive DNA sequences, 264
transcription of, 222
in translation, 228, 229, 230
wobble, 226 Transformation, 200-201, 247 transformer (tra) gene, 934 Transforming growth factor- β (TGF- β), 371
Transgenic animals
a-Tantitrypsin production in,
326 silk protein production in, 311 Transgenic organisms
public concerns with, 327-328 qualities of host organisms, 314 Transgenic plants
improved nutritional characteristics, 327 insecticide expression in,
325-326 resistance to herbicides, 327 Transition-state species, 103 Translation, 221
blocking with antisense RNA,
322 elongation cycle, 228-229, 229 eukaryotic, 261 genetic code and, 223-225 initiation complex, 228-229 overview of, 225
point mutations and, 234-235 posttranslational events,
231-233 regulation of, 230-231 start and stop signals in, 229,

230 termination of, 229-230 tRNA in, 225-226, 227, 228, 229, 230 Translational repressor proteins, 277 Translocations, 173, 236, 335 Transmissible spongiform encephalies (TSEs), 334 Transmission electron microscopy, 57 Transpiration antitranspirants, 628 stomata and, 627-628 transpiration-cohesion-tension mechanism, 625-626 in xerophytes, 685-686, 690 Transplant rejection, 368 Transporter proteins, genetic diseases of, 333-334 Transposable elements, 249, 264-265 Transposons, 249, 257, 264-265, 316 Transverse tubules, 838-839 Tra protein, 934 Tree ferns, 513 Tree squirrels, 927 Trematoda, 554, 578 *Treponema pallidum*, 471, 749 *Triaenodon obesus*, 585 Triassic period, 380-381, 389, 392 Tricarboxylic acid cycle. See Citric acid cycle *Trichinella spiralis*, 567 Trichinosis, 567 Trichocysts, 486 Trichoderma, 988 Trichoptera, 574 *Tridacna gigas*, 561 *Trifolium repens*, 409, 976, 977 Triglycerides, 49-51 Inlobites, 568 -*Trimeresurus sumatranus*, 590 Tri-methyl amine oxide, 917 Triose phosphate, 147 Triose phosphate dehydrogenase, 119, 121 Triple bonds, 23 Triplet repeats, 339 Triploblastic animals, 545 Trisomy, 172, 173, 442 Triticum, 527 *T. aestivum*, 167 Tritium, 381 tRNA. See Transfer RNA Trochophores, 553 Trophic levels, 995-996 Trophoblast, 758, 768 Tropical forests deciduous, 1024 deforestation rates, 1031 evergreen, 1025 fragmentation in, 1036 Tropomyosin, 276, 837, 838, 839 Troponin, 837, 838, 839 Troposphere, 1000 trp operon, 252-253 True-breeding, 178 True bugs, 572 Truffles, 536 Trumpet cells, 489 *Trygon pastinaca*, 585 *Trypanosoma*, 483, 484 Trypanosomes, 483, 484 Trypsin, 900, 901 Trypsinogen, 900 Tryptophan, 37, 224, 889, 890 trp operon, 252-253 *Tschermak*, Erich von, 178 *Tsien*, Joe, 791 T tubules, 838-839 Tuataras, 589, 590 Tubal ligation, 746, 747 Tubal pregnancy, 758 Tube cell, 668, 669 Tube feet, 579 Tuberculosis, 256, 372, 472 Tubers, 605, 606, 676 *Tubulanus polymorphus*, 557 Tubular guts anatomy of, 894-895 digestive enzymes and, 896 endosymbiotic bacteria and, 895-896 Tubular hearts, 867 Tubulin, 74, 276 Tumor cells. See Cancer cells Tumors creation of, 342 gene amplification and, 275 totipotency in, 299 types of, 342 See also Cancer Tumor suppressor genes, 344-345, 346 Tundra, 1016 Tunicates, 582-583 Turbellarians (*Turbellaria*), 554, 578 Turbinaria, 551 Turgidity, 621 Turgor pressure, 87, 621 Turner syndrome, 194 Turnover, 1000 *Tursiops truncatus*, 594 Turtles, 589-590 Twins, 743, 759 Twin studies, 189-190 Twitches, 839-840 Two-peaked distributions, 406 Tympanic membrane, 802, 803, 804 Type 1 diabetes. See Insulin-dependent diabetes Typhus, 256

Tyrosine, 37, 224, 332

Tyrosine kinase receptors, 283, 287

Ubiquinone (Q), 125, 126, 127

Ubiquitin, 277

Ulcers, 898-899

Ulna, 843

Ulothrix, 493

Ultraviolet radiation, 139

detection in insects, 812

mutation effects, 236 *Ulva latum*, 492-193 Umbels, 522 Umbilical cord, 768, 771 Umbilicus, 771 Underground stems, 676 Undernourishment, 132, 888-889 Uniport transporters, 89 Uniramians (Uniramia), 571-574,

578 United States

age distribution in, 961-962

geographic distribution of endangered species, 2042

proportion of species extinct or at risk, 1034 Unsaturated fatty acids, 50 Unsegmented worms, 566-567 Upregulation, of hormone receptors, 729 Upwellings, 999 Uracil, 47, 48

deamination, 236, 237

genetic code, 224

in RNA, 220

spontaneous formation in DNA, 338 Urbilateria, 552 Urea, 912

Uremic poisoning, 922 Ureotelic animals, 912 Ureter, 919 Urethra, 739, 740, 919 Uric acid, 912, 913-194, 915 Uricotelic animals, 912 Urinary bladder, 919 Urination, 919 Urine, 911

of annelids, 914

concentrating in the kidney, 920-921

of flatworms, 914

human, 913-194

renal tubules and, 916 Urochordata, 578, 582 *Uroctomis monax*, 570 Urodela, 587 *Ursus maritimus*, 887 *Urticina lofotensis*, 548 Uterine cycle, 743-745

Uterus, 739

blastocyst implantation, 742,

745, 758, 769 egg in, 741 uterine cycle, 743-745

Vaccination, 359, 360 Vaccine proteins, 323 Vacuoles

functions of, 63, 71-72

in protists, 480, 481

See also Plant vacuoles Vagina, 737, 741, 746 Valine, 37, 224, 889, 890 Vampire bats, 910 Van der Waals forces, 21, 25, 40 Variable number of tandem repeats (VNTRs), 328 Variable region, of immunoglobulins, 362, 363, 369 Varicose veins, 878 Vasa recta, 920 Vascular bundles, 615 Vascular cambium, 613, 615-616 Vascular endothelial growth factor, 714 Vascular rays, 615-616 Vascular system (animal)

arteries and arterioles, 875-876

capillaries, 876-877

cardiovascular disease and, 878-879

lymphatic vessels, 877

veins, 877-878

See also Blood vessels; Circulatory systems Vascular tissue system (plant), 608, 609, 610-611

in *Ainborclla*, 525

in gymnosperms, 518

in leaves, 618

in plant evolution, 502-503, 507

in roots, 614

secondary growth, 613, 615-617

in stems, 615 Vas deferens, 739, 740, 747 Vasectomy, 746, 747 Vasopressin, 718-719, 882, 883. See also Antidiuretic hormone Vectors

artificial chromosomes, 316

expression vectors, 322-323

for plants, 316

plasmids, 315, 316

properties of, 315

viruses and, 242-243, 316 Vegetal hemisphere, 301, 755, 756

in cleavage, 757

developmental potential, 761 Vegetarianism, 889-890 Vegetative organs, 603-606 Vegetative reproduction, 165

in agriculture, 677

forms of, 676-677

See also Asexual reproduction Veins (animal), 868, 875, 877-878.

See also Vascular system Veins (plant), 618

Venter, Craig, 255

Ventilation, 853

in bird lungs, 854-856

in fishes, 854

tidal breathing, 856-857

Ventral horn (spinal cord), 817

Ventricle

human heart and cardiac

cycle, 871-872, 873-874 in reptilian hearts, 870 in three-chambered hearts, 869 in two-chambered hearts, 869

Ventricular fibrillation, 874

Ventromedial hypothalamus, 906

Venules, 868

Venus flytraps, 634, 644

Vernalization, flowering and, 675-676

Vertebral column, 583, 843

Vertebrates (Vertebrata), 583 axis determination in, 309 body temperature regulation,

707-709 central nervous system,

774-775 circulatory systems, 868-871 colonization of land, 587-591 dorsal-ventral development,

543 equilibrium organs in, 801-803 eye anatomy and function,

807-812 eye development, 301-302 generalized body plan, 583 nephron structure and function, 915-916, 927 notochord and, 430, 432 origin of, 583-586 phylogeny, 428[^]130, 584 skeleton and joints, 843-846 teeth, 894 tetrapods, 587

Vertical transmission, 243, 244

Very-low-density lipoproteins (VLDLs), 904, 905

Vesicles

from endoplasmic reticulum,

66 in Golgi apparatus, 65, 66 in protists, 480-481

Vessels/Vessel elements, 521, 608, 609

Vestibular apparatus, 801, 802, 803

Vestigial organs, 902

Vestimentiferans, 559

Vibrio cholerae, 467

Vicariant distributions, 1009, 2020, 1027

Victoria (Queen of England), 196

Vicunas, 861-862

Villi, 896

Vincristine, 347

Viral retrotransposons, 265

Virginia opossum, 594

Virions, 240, 241

Viroids, 240, 244

Viruses

cancer-causing, 343 characteristics of, 240-241

classifications of, 241

discovery of, 240

as DNA vectors, 316

enveloped, 243

genome, 260

Hershey-Chase experiment, 201-202

regulation of gene expression in, 254, 255

reproductive life cycles, 241-243

size range, 240

temperate, 242

transduction and, 247

using in genetic research, 239-240

virulent, 242 Visceral mass, 560 Visual communication, 935-936 Visual cortex, 823-824 Visual disparity, 824 Visual systems

binocular vision, 824
 detection of ultraviolet light, 812
 in invertebrates, 807
 rhodopsin and photosensitivity, 805-807
 vertebrate eye, 807-812
 visual cortex, 823-824 Vitamin A, 52, 892
 deficiency, 327 Vitamin B, 892, 893
 neural tube defects and, 766 Vitamin C, 891, 892 Vitamin D, 52, 891, 892 Vitamin E, 52, 892 Vitamin K, 52, 892, 902
 Vitamins, 52, 891-892 Vitelline envelope, 753, 754, 755 Vitis vinifera, 677 Viviparity, 739 VNTRs, 328 Voice box. See Larynx
 Volatinia jacarina, 416 Volcanoes, 383-384, 388, 389,
 1004 Voltage, 777 Voltage-gated channels
 in action potentials, 780, 781, 782, 783
 defined, 778
 in neurotransmitter release, 786, 787
 in pacemaker cells, 873
 in phototransduction, 806, 807
 in sensory transduction, 795, 796 Voluntary nervous system, 815 Volvox, 492
 von Frisch, Karl, 928, 936 vp corn mutants, 660 Vries, Hugo de, 178 Vulpes macrotis, 707 Vulva, in C. elegans, 302, 303
 Waggle dance, 936 Walking sticks, 572 Wallace, Alfred Russell, 2, 397 Warblers, 419, 420 Warty chameleon, 2042
 Washington, t.i'o;
 Wasps "4 spatial learning, 928 929 See also \\\ menopterans
 Water absorption in large intestine,
 901-902 a< id properties, 29 cohesive strength dissoh ing ionic solids in, 25 dynamics of movement in
 capillaries ^^-\$77 heat ol e\ aporation, 707 heat ol vaporization, 27-28 hydrogen bonds and, 23, 27 hydrological cycle, 1001
 osmosis and, 86-87, 621 in photosynthesis, 136,137 polar covalent bond, 23 specific heat, 27, 993 structure and properties,
 26-28 surface tension, 28 uptake and transport in plants, 620-626
 Water balance, tissue fluids and, 910-911
 Water bears, 568, 578
 Water breathers
 effects of temperature on gas
 exchange, 850-851 evolutionary transitions to air
 breathing, 869 partial pressures of carbon dioxide and, 852
 Water ferns, 640
 Water molds, 477, 490
 Water potential, 621, 650
 Water-soluble hormones, 728-729
 Water-soluble vitamins, 892
 Water vascular system, 579
 Watson, James, 202, 203, 204
 Wavelength, 138-139

Waxes, 52-53

Weaverbirds, 956, 957

Webs. See Spider webs

Weedy sea dragon, 586

Weevils, 1010

Wegener, Alfred, 1008

Weight, distinguished from

mass, 19n Weinberg, Wilhelm, 400 Welwitschia, 518, 519, 521

Went, Frits, W., 653, 654 Wernicke's area, 828, 829 Western tent caterpillar, 978-979 Wetland restoration, 1043 Whales, 887
baleen, 577

communication in, 936

evolution of, 385

nasal passages, 752

overexploitation of populations, 970, 971

piloting in, 940 Whale shark, 585 Wheat

chromosome pairs, 267

polyploidy, 173

vernalization and, 675-676 Whip grafting, 677 Whiptail lizards, 734 Whisk ferns, 501, 512-513 White blood cells, 259, 304, 354,

355 White-crowned sparrows,

929-930 Whitefish, 962, 991 White-fronted bee-eaters, 954 White matter, 817 White muscle, 840-841 White-plumed
antbird, 1036 White-spotted anemone, 548 Whorls, floral, 304 Widowbirds, 950-951 Wiesel, Torsten, 823 Wigglesworth,
Vincent, 715-716 Wildebeest, 957 Wild mustard, 399 Wild type alleles, 186 Wilkins, Maurice, 203 Willow, 680 Wilm's tumor,
345 Wilmut, Ian, 298 Wilson, Edmund, 1012 Windpipe. See Trachea Wind pollination, 667 Wine grapes, 677

Winged insects, 572, 573, 574 Wingless insects, 572 Wings

convergent and parallel evolution in, 427

in insects, 572 Wintergreen, oil of, 681 Winter wheat, 675-676 Wobble, 226 Wolf spiders, 570 Womb, 741. See also Uterus
Wood, 518, 613, 615, 616-617 Wood ticks, 570

Xanthium, 665 X chromosomes

Barr bodies and inactivation, 274-275

functions, 194

sex determination, 194

sex-linked inheritance, 195-196 Xenopus, 761

Xerophytes, 685-687, 688-689 Xiphosura, 569 XIST gene, 275 X-linked diseases, 335 X ray crystallography, 202-203,
440 X rays, mutation and, 236 Xylem, 503, 507, 608, 609, 610

of Amborella, 525

apoplastic and symplastic paths to, 622-624

in gymnosperms, 518

in roots, 614

secondary, 615, 636, 617

transpiration-cohesion-tension mechanism, 625-626

transport of water and minerals in, 621, 624-626

in vascular bundles, 615

vascular cambium and, 613 Xylem sap

measurement of pressure in, 626

transport of, 624-626

Yams, 432-433

Yarrow's spiny lizard, 948

Y chromosome functions of, 194 sex determination and, 194 sex-linked inheritance and, 196

Yeast artificial chromosome (YAC), 316

Yeasts, 530, 531

artificial chromosome, 316 DNA rearrangements in, 275 genome, 261, 262 hemiascomycete, 535-536 mating types, 275 as transgene hosts, 315

Yellowstone-to-Yukon Initiative (Y2Y), 1042

Yellowtail snapper, 586

Yellow water lily, 688

Yersinia pestis, 467

Yews, 520-521

Yolk, 756

gastrulation and, 763 influence on cleavage, 757

Yolk sac, 767-767

Yucca brevifolia, 986

Yucca plants, 985-986

Zebra mussels, 1037 Zebras, 1009 Zeevaart, Jan A. D., 675 Zinc

in animal nutrition, 890

in plant nutrition, 636 Zinc deficiency (plant), 636 Zinc finger motif, 273 Z lines, 836, 837 Zona pellucida, 753, 754, 758 Zones of upwelling, 999 Zooids, 960 Zoospores, 490, 534 Zygomycetes (Zygomycota), 530,

533, 534-535 Zygosporangium, 530, 535 Zygosporangium, 530, 535 Zygotetes, 165, 736

blastocyst stage, 758

egg and sperm contributions to, 754-755

totipotency of, 296 Zymogens, 896, 899, 900

About the Book

Editor: Andrew D. Sinauer

Project Editor: Carol J. Wigg

Developmental Editor: James Funston

Review Coordinator: Susan McGlew

Copy Editor: Norma Roche

Production Manager: Christopher Small

Book Layout and Production: Janice Holabird, Jefferson Johnson, and Joan Gemme

Art Editing and Illustration Program: J/B Woolsey Associates

Design: Jefferson Johnson

Book Cover Design: Jefferson Johnson

Photo Research: David McIntyre

Index: Grant Hackett

Color Separations: Vision Graphics, Inc. and Burt Russell Litho

Cover Manufacture: Henry N. Sawyer Company, Inc.

Book Manufacture: Courier Companies, Inc.



ICONS 'Continued twin inside

TOPIC

\ Generalized Rower Vh\ logenj ol the Fungi A gomj cetes Reproduce

Sexuall) In Fusion of

[Wo Gametangia rhe ! Lfe Cycle of a

Euascomycete Cnidarian Life Cycles Sumtnar)

General Characteristics of

the Major Protostomate Phyla

32 575 Summary

33 " L < A Probable Deutero-stomate Phylogeny

33 588 In and Out of the Water 33 589 An Egg for Dry Places

33 600 Summary

34 614 Root Anatomy 34 615 Vascular Bundles in Stems 34 616 Vascular Cambium

Thickens Stems and Roots

34 618 The Eudicot Leaf

35 623 Apoplast and Symplast

35 630 The Pressure Flow Model

36 637 Identifying Essential Elements for Plants

36 643 The Nitrogen Cycle

648 Patterns of Early Shoot Development

36 651 Embryos Mobilize Polymer Reserves

653 The Darwins' Photo-tropism Experiment

37 654 Plants Respond to Light and Gravity

38 666 Development of Gametophytes and Nuclear Fusion

38 668 Pollen Nuclei and Double Fertilization

38 669 Early Development of a Eudicot

680 Signaling between Plants and Pathogens

39 690 Summary

40 698 The Major Organ Systems of Mammals

40 698 Physiological Regulation and Homeostasis

40 706 Environmental Temperature and Mammalian Metabolic Rates

41 714 The Endocrine System of Humans

47 845

48 856

48 858

48 859

48 861

TOPIC

Complete Metamorphosis Principal Hormones of

Humans The Reproductive Tract of

the Human Male Seminiferous Tubules Are

the Site of Spermatogenesis The Reproductive Tract of

the Human Female The Ovarian Cycle The Uterine and Ovarian

Cycles The Acrosomal Reaction The Slow Block to

Polyspermy Gastrulation in the Frog

Xenopus The Extraembryonic

Membranes The Course of an Action

Potential

Synaptic Transmission Begins with the Arrival of an Action Potential

Some Well-Known Neurotransmitters

Structures of the Human Ear

Eyes Like Cameras

The Retina

What Does the Eye Tell the Brain?

The Spinal Cord Receives and Processes Information from the Body

The Reticular System

The Human Cerebrum

Language Areas of the Cortex

The Structure of Skeletal Muscle

T Tubules in Action

The Release of Ca^{2+} from

the Sarcoplasmic

Reticulum Triggers

Muscle Contraction Types of Joints The Path of Air Flow

through Bird Lungs The Human Respiratory

System Into the Lungs and Out

Again Oxygen-Binding

Adaptations

TOPIC

The Oxygen-Binding Properties of Hemoglobin Can Change

Summary

Vertebrate Circulatory Systems

The Human Heart and Circulation

The Cardiac Cycle

Anatomy of Blood Vessels

Mineral Elements Required by Animals

Vitamins in the Human Diet

The Human Digestive

System Fuel Metabolism Is

Controlled by Hormones Metanephridia in

Earthworms The Vertebrate Nephron The Human Excretory

System Concentrating the Urine

The Waggle Dance of the

Honeybee The Time-Compensated

Solar Compass Bluegills are Energy

Maximizers Summary Patterns of Population

Growth Population Growth Is

Influenced by the

Carrying Capacity Exponential and Logistic

Population Growth Density-Dependent

Factors Regulate

Population Size Types of Ecological

Interactions Predator-Prey

Interactions A Rain Shadow Energy Flow through an

Ecosystem Biogeochemical Cycles Summary Major Biogeographical

Regions Terrestrial Biomes The Value of Corridors Summary

Following is a directory of the Experiment and Research Method figures in LIFE.

FIGURE

TOPIC AND PAGE

FIGURE

TOPIC AND PAGE

FIGURE

TOPIC AND PAGE

EXPERIMENTS

1.14 An Experiment Demonstrates that

Parasites Influence Amphipod

Behavior 13 5.7 Diffusion Leads to Uniform

Distribution of Solutes 86 7.74 Two Experiments Demonstrate

the Chemiosmotic Mechanism

128 8.2 Water Is the Source of the Oxygen

Produced by Photosynthesis

137

37.14 37.15 37.18 37.21 38.12 38.13

38.15 38.16

39.5

Auxin and Leaf Abscission 655 Auxin and Apical Dominance 656 Auxin Affects Cell Walls 657 Sensitivity of Seeds to Light
661 Night Length and Flowering 672 The Effect of Interrupted Days

and Nights 673 Plants Can Measure Time 674 Evidence for a Flowering

Hormone 675 Overcompensation for Being

Eaten 682 Metabolic Compensation 701 The Hypothalamus Regulates

Body Temperature 708 Adjustable Set Points 709 A Diffusible Substance Triggers

Molting 715 Vegetal Pole Cells Contain Essential Cytoplasmic Factors 760 Spemann's Experiment 762 Tissue Transplants
Reveal the

Process of Determination 763 Repeated Stimulation Can Cause

Long-Term Potentiation 792 What Does the Eve Tell the Brain?

811 Receptive Fields of Cells in the

Visual Cortex 823

The Conditioned Eye Blink Reflex

Depends on a Cerebellar

Circuit 827 Smooth Muscle Action 835 The Path of Air Flow through

Bird Lungs 856 Releasing the Pecking Response

928 Spatial Learning 929 Two Critical Periods for Song

Learning 930 Hormonal Control of Sexual

Behavior 931 Circadian Rhythms 938 Where the Clock Is 939 Distance-and-Direction

Navigation 942 Star Patterns Can Be Altered in a

Planetarium 943 The Costs of Defending a

Territory 948

J

Bluegills are Energy Maximizers

949 The Longer the Tail, the Better the

Male 950 Flocking Provides Defense against

Predators 953 Plants Compete with their Roots

and Shoots 977

55.4 Microparasites Can Cause Population Crashes 978

55.6 Prey Population Cycles May

Have Multiple Causes 980

55.7 Predators Exclude Prey from

Some Habitats 981

55.14 An Experiment Demonstrates the

Benefits of Housing Ants 984

56.15 Increased Atmospheric Co₂

Concentrations Alter Soil Communities 1003 58. 11 An Experiment Demonstrates the Value of Corridors 1036

RESEARCH METHODS

4.3 Looking at Cells 57

5.3 Membrane Proteins Revealed by

the Freeze-Fracture Technique

81

10.1 A Controlled Cross between Two

Plants 177 7 7.9 Density Gradient Centrifugation

206' 7 7.20 Sequencing DNA 215 7 7.2 7 The Polymerase Chain Reaction

216

13.6 Growing Bacteria in the

Laboratory 245

74.7 Nucleic Acid Hybridization 266 7 7.2 Gel Electrophoresis 313

7 7.3 A Hybridization Probe 314

77.8 Constructing a Gene Library 318

17.10 Making a Knockout Mouse 320

78.6 Isolating Human Genes 336

78.7 RFLP Mapping 337

78.7 7 DNA Testing by Allele-Specific

Cleavage 342 78.72 DNA Testing by Allele-Specific Oligonucleotide Hybridization 342

78.2 7 Two Approaches to Sequencing

DNA 349 19.13 Creating Hybridomas for the Production of Monoclonal Antibodies 365

24.3 Amino Acid Sequence Alignment

440

35.8 A Pressure Bomb 626

44.5 Measuring the Resting Potential 777

44.8 The Nernst Equation 779

44.72 Patch Clamping 783

48. 11 Measuring Lung Ventilation with

a Spirometer 857 57.7 Experimental Island Biogeography 1013

www.thelifewire.com

